Molecular Systematics and Structure, Development and Evolution of Floral Asymmetry in the Genus *Senna* (Leguminosae)

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To my mother and my father.

A mia madre e a mio padre, che mi hanno visto partire per il mondo.

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The true delight is in the finding out rather than in the knowing.
Isaac Asimov

PREFACE

I first came into contact with the subject of my thesis in 2000. I was studying at the University of Zürich, and I was doing a practical work for my *Grosses Nebenfach* (main optional subject) at the Botanical Garden of Geneva. My big motivation to start a Diploma thesis in Botany was actually the opportunity to travel into the subtropics and to experience what it means to do research in botany. When I asked Prof. Rodolphe Spichiger, of the Conservatoire and Jardin Botaniques of Geneva, to do a Diplomathesis on some South-American group of plants, I had not yet had started my studies of Systematic Botany. A taxonomic treatment of the genus *Senna* for the Flora of Paraguay, has been his suggestion. He strongly recommended to ask Prof. Peter Endress, of the Institute of Systematic Botany, to be co-supervisor of my work, and to carry out a morphological study on the flowers of *Senna* with him. At that time, however, I did not know Peter, neither other persons of the Institute… Despite that I was immediately very glad that he would be my supervisor!

In January 2001, as part of my Diploma thesis, I travelled to Argentina and not to Paraguay, since I was told, "Argentina is a safer place than Paraguay". I visited several herbaria and went on two collecting trips with botanists of the Instituto de Botánica del Nordeste in Corrientes (N-Argentina). When I saw the first *Senna* species, I was amazed by its beauty and I immediately fell in love with these plants! Not only did I become very enthusiastic about *Senna*, but also about the country I was visiting, and about the *Castillano* (Latin-American Spanish), the new language I was learning. This botanical experience became an exepcional experience of life.

As I started to explore the flower morphology in the collected *Senna* species, I realized that there was something special and intriguing. Peter already knew about the interesting floral features of the genus, but he did not expect such a diversity. Therefore, he proposed to focus on the taxonomy and leave the morphological part for a potential doctoral project. So I did.

My PhD project started in December 2002 in a big stress, since the entire project depended on what floral material I could find on my collecting trips. Because most *Senna* species occur throughout the American continent, in two months, I planned field trips in several countries of Latin-America; a challenging task! The two long oversea journeys turned out to be a fantastic and memorable experience: I visited Argentina (once more), Brazil, Mexico, Panama, and even Paraguay. By the way, I cannot remember how many times I was asked: "Did you choose your plant group according to the countries you wanted to visit? Or…"

GENERAL INTRODUCTION

BACKGROUND

Symmetry has been studied in all its facets since antiquity, especially in architecture, arts, mathematics, physics, and, more recently, also in biology. In botany, Sprengel (1793) was probably the first researcher interested in floral symmetry. He grouped flowers into two categories: 'regular', i.e. with more than one symmetry plane, such as a bell-flower, or 'irregular', i.e. with only one symmetry plane, such as an orchid. Today, Sprengel's terms have been replaced by the conventional 'polysymmetric' ('actinomorphic') and 'monosymmetric' ('zygomorphic') (see Endress 1999).

In flower-pollinator interactions, floral symmetry is a specific cue that appears to signal a high quality and/or quantity of nectar or pollen to insects which, in turn, may exert strong selection pressure on symmetric elements in flowers (e.g., Giurfa et al. 1999). When considering this preference of pollinating insects for symmetric flowers, it is not surprising that most angiosperms actually have polysymmetric or monosymmetric flowers. Asymmetric flowers are rare, and thus intriguing for researchers.

Floral asymmetry mainly occurs in a few taxa of large families or orders with prevailing floral monosymmetry (e.g., Lamiales, Leguminosae, Orchidaceae, Zingiberales), and only exceptionally in basal angiosperms (e.g., Winteraceae; Endress 1999). Particularly interesting is enantiomorphy, a special kind of floral asymmetry in which there are two morphs and flowers of the left and right morph mirror one another. For example, the style is deflected either to the left or to the right of the median plane, a condition known as enantiostyly, which occurs in at least ten angiosperm families of both monocots and eudicots (Jesson 2002). Although enantiostylous flowers have fascinated researchers for a long time (e.g., Todd 1882), little is known about their development (Jesson et al. 2003b; Tucker 1996, 1999), genetics (Jesson and Barrett 2002a, b), role in pollination biology (e.g., Delgado Salinas and Souza Sánchez 1977; Dulberger 1981; Gottsberger and Gottsberger-Silberbauer 1988), or evolution (Harrison et al. 1999; Jesson et al. 2003a; Jesson and Barrett 2005).

Enantiostylous flowers are nectarless and are visited by pollen-collecting bees that vibrate the stamens to extract and collect the pollen to feed their larvae (buzz pollination; Buchmann 1974). Buzz-pollinated flowers typically have androecia characterized by different kinds and degrees of structural specialization associated with the unusual pollination mode, including poricidal anther dehiscence (i.e. dehiscence by apical pores) and heteranthery (different kinds of stamens with separation of 'feeding' and 'pollinating' function) and, in enantiostylous flowers, not only deflection of the style but also deflection of stamens to the side opposite the style (Buchmann 1983). While bees buzz the flowers, the thecae act as a resonating chamber, in which pollen grains bounce when vibrated, gaining kinetic energy, until they are forced through the apical pore (Buchmann and Hurley 1978). Electrostatic forces seem to play an important role in buzz pollination, promoting both adhesion of the pollen grains on the hairy insect body and, subsequently, "jumping" from there to the stigma of another flower (e.g. Corbet et al. 1982, 1988).

Although bees groom the pollen from their bodies, there is pollen remaining on their body, especially in parts the bees cannot reach with their legs (e.g. Buchmann 1983). In enantiostylous flowers, pollen of a left floral morph is ideally positioned on a place of the insect body that corresponds to the position of the stigma in a right floral morph, and vice versa. Enantiostyly has thus commonly been regarded as a device to promote outcrossing (Todd 1882). When both left and right floral morphs are on the same plant, enantiostyly may actually promote geitonogamous self-pollination between different morphs. Nevertheless, Jesson et al. (2003a) and Jesson and

Barrett (2005) provided evidence for the hypothesis that, compared to monosymmetry, enantiostyly functions to reduce geitonogamous pollen transfer with a concomitant increase in pollen export. Other researchers have suggested that enantiostyly may facilitate the access of the pollen-collecting bees to the stamens, which the bees clasp and vibrate, forcing them to adopt a position that results in more efficient pollen removal (Westerkamp 2004), and also may protect the gynoecium from damage by buzzing bees (Dulberger 1981).

Knowledge on the functional significance of enantiostyly allows us to suggest and interpret hypotheses on the evolution of floral asymmetry, but detailed knowledge on the structural diversity of enantiostylous flowers is a precondition to accurately infer hypotheses of floral asymmetry evolution. Floral symmetry is composed of several structural elements, as are pollination syndromes. Flowers with different floral asymmetries may have originated through different evolutionary and developmental pathways, and thus the asymmetries may not be homologous. Therefore, the elements involved in the floral symmetry should be treated individually if used for phylogenetic hypotheses (Bruneau 1997; Herendeen et al. 2003) or optimization studies (Marazzi et al. 2006). Detailed structural studies are fundamental to identify these elements.

SENNA: AN IDEAL STUDY GROUP

Senna (Leguminosae, Cassiinae), formerly known as *Cassia* s.l., is a large genus of herbs, shrubs, treelets, tall trees, and lianas, which have successfully colonized a wide range of habitats. About 80% of the approximately 350 species ascribed to the genus occur on the American continent, while most of the remaining members are found in the tropics and subtropics of the other continents (Irwin and Barneby 1982; Randell and Barlow 1998). *Senna* is absent from Europe, although several species have long been used in European medicine (e.g., Colladon 1816). The current classification system of *Senna* recognizes six sections (*Astroites*, *Chamaefistula*, *Paradictyon*, *Peiranisia*, *Psilorhegma*, and *Senna*) and reflects traditional interpretations of floral morphological evolution within the genus (Irwin and Barneby 1982; Randell 1988, 1989, 1990; Singh 2001).

Flowers are highly diverse and interesting in *Senna*. The usually yellow flowers are buzz-pollinated, and there are both species with monosymmetric, and species with asymmetric, enantiostylous flowers, in which the entire carpel is deflected, and, in addition, stamens and petals may contribute to the floral asymmetry (Irwin and Barneby 1982; Marazzi et al. 2006). Furthermore, flowers of *Senna* display an array of other floral traits typically related to buzz pollination, such as heteranthery, poricidal anther dehiscence with unusual, long and tubular anther tips in some species, and extremely point-tipped stigmas. Due to its outstanding diversity (e.g., Venkatesh 1957), the androecium of *Senna* is probably one of the best examples to illustrate the specializations that buzz flowers have undergone in conjunction with their pollination mode (Marazzi et al., submitted).

The diversity of floral structures and symmetry patterns raises interesting questions on the evolution of floral morphology in *Senna*. Conversely, it makes *Senna* an outstanding study group to investigate floral structural specialization and asymmetry associated with buzz pollination in a phylogenetic framework. This has been the challenge and motivation in the present thesis.

AIMS OF THIS THESIS

The major aim of **part 1** (Marazzi et al. 2006) is to generate a molecular phylogeny of the genus *Senna* to be compared with current classification systems and traditional interpretations of floral morphological evolution within the genus (Irwin and Barneby 1982; Randell 1988, 1989, 1990; Singh 2001). More specifically, the monophyly of the sections *Chamaefistula*, *Peiranisia*, *Psilorhegma*, and *Senna*, as defined by Irwin and Barneby (1982) is tested, and the position of the monotypic *Astroites* and *Paradictyon* is clarified. The morphological traits that are congruent with

the clades defined by the molecular phylogeny are explored, and whether the phylogenetic tree supports the hypotheses of floral evolution proposed by Irwin and Barneby (1982). In this part, also the evolution of extrafloral nectaries (i.e. secretory structures on leaves), which are another characteristic feature of many species of *Senna* is explored.

In **part 2** (Marazzi et al., submitted), the structural diversity of the androecium and gynoecium in *Senna* is investigated, focusing on the structural specialization in anthers and stigmas associated with buzz pollination, but not involved in floral symmetry. In particular, patterns of heteranthery, patterns in the diversity of stamens and anther dehiscence, and presence of undescribed stigma types are identified. The results are used to recognize synapomorphies based on the new infrageneric relationships indicated by the molecular phylogenetic studies (Marazzi et al. 2006), and to test whether they support traditional interpretations on the evolution of the androecium by Irwin and Barneby (1982). Finally, it is discussed, how the implications of the results on anther and stigma diversity can improve our understanding of pollination and floral evolution in the genus.

The aim of **part 3** is to dissect the floral symmetry syndrome in *Senna* into its component structural elements and differentiate patterns of homology, especially in asymmetric flowers. I focus on the following aspects: patterns of floral asymmetry, patterns in the diversity of petal form and venation, synapomorphies congruent with the infrageneric relationships supported by the molecular phylogenetic studies (Marazzi et al. 2006), and floral development of species with different patterns of floral asymmetry. In addition, the implications of these results to improve our understanding of pollination and floral symmetry evolution in *Senna* are discussed.

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PART 1

MOLECULAR PHYLOGENY OF *SENNA*

Collecting *Senna macranthera* in Argentina.

PHYLOGENETIC RELATIONSHIPS WITHIN *SENNA* (LEGUMINOSAE, CASSIINAE) BASED ON THREE CHLOROPLAST DNA REGIONS: PATTERNS IN THE EVOLUTION OF FLORAL SYMMETRY AND EXTRAFLORAL NECTARIES

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ABSTRACT

Senna (Leguminosae) is a large, widespread genus that includes species with enantiostylous, asymmetric flowers and species with extrafloral nectaries. Clarification of phylogenetic relationships within *Senna* based on parsimony analyses of three chloroplast regions (*rpS*16, *rpL*16, and *matK*) provides new insights on the evolution of floral symmetry and extrafloral nectaries. Our results support the monophyly of only one (*Psilorhegma*) of the six currently recognized sections, while *Chamaefistula*, *Peiranisia*, and *Senna* are paraphyletic, and monotypic *Astroites* and *Paradictyon* are nested within two of the seven major clades identified by our molecular phylogeny. Two clades (I, VII) include only species with monosymmetric flowers, while the remaining clades (II–VI) contain species with asymmetric, enantiostylous flowers, in which either the gynoecium alone or, in addition, corolla and androecium variously contribute to the asymmetry. Our results further suggest that flowers were ancestrally monosymmetric with seven fertile stamens and three adaxial staminodes, switched to asymmetry later, and reverted to monosymmetry in clade VII. Fertility of all 10 stamens is a derived state, characterizing the *Psilorhegma*-subclade. Extrafloral nectaries evolved once and constitute a synapomorphy for clades IV–VII ("EFN clade"). These nectaries may represent a key innovation in plant defense strategies that enabled *Senna* to undergo large-scale diversification.

KEY WORDS

Cassiinae; extrafloral nectaries; floral asymmetry; key innovation; *matK* gene; *rpL*16 intron; *rpS*16 intron; *Senna*.

INTRODUCTION

Formerly included in *Cassia* L., *Senna* (Leguminosae, Caesalpinioideae, Cassiinae) is a large, widespread, and diverse genus characterized by a distinctive floral morphology and the presence of extrafloral nectaries (referred to as EFNs from here on) in numerous species. *Senna* displays a high diversity of habits, including herbs, shrubs, treelets, tall trees, and lianas, and has successfully colonized a wide range of habitats in different climates and latitudes. Of the approximately 350 species currently ascribed to the genus, 80% occur on the American continent, while most of the remaining members are found in tropical Africa, Madagascar, and Australia, with only a few species in southeastern Asia and some on the Pacific Islands (Irwin and Barneby 1982; Randell and Barlow 1998). No *Senna* species are native to Europe, although several of them have long been used in the European medical tradition (e.g., Colladon 1816).

Shifting taxonomic boundaries mark the history of traditional systematic treatments of *Senna*. These shifts are best explained by the difficult taxonomic interpretation of morphological variation in *Senna*. For example, the high degree of specialization typical of the buzz-pollinated *Senna* flowers complicates the identification of traits that can be unambiguously used for taxonomic purposes. The yellow, nectarless flowers offer pollen as a reward to their pollinators, usually large female bees of different genera, for example, *Xylocopa* (Dulberger 1981; Irwin and Barneby 1982; Gottsberger and Silberbauer-Gottsberger 1988). The heterantherous flowers of *Senna* generally have 10 stamens; the three adaxial stamens are typically staminodial, while the remaining seven, or fewer, are fertile. The fertile stamens are poricidal and differentiated into two sets: one set of four middle stamens (between the adaxial staminodes and abaxial stamens; see also fig. 3A), which bees buzz to extract food pollen, and a second set of two or three (often longer) abaxial stamens, whose pollen is deposited on the bee's body during buzzing and transported to the stigma of other flowers (Buchmann 1974; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003). Furthermore, many species of *Senna* have asymmetric flowers, with the gynoecium deflected either to the left or to the right within the same inflorescence (Dulberger 1981; Irwin and Barneby 1982; Gottsberger and Silberbauer-Gottsberger 1988). This type of floral asymmetry is known as enantiostyly. In many enantiostylous *Senna* species, corolla and androecium additionally contribute to the floral asymmetry.

Typically found on leaves and rarely also on pedicels, EFNs are another characteristic feature of many *Senna* species. They occur in ca. 76% of the American species (Irwin and Barneby 1982), numerous Australian species, more rarely in African species, but apparently in no Southeast Asian species. These organs attract ants, which feed on the nectar and protect the plant against herbivores, thus forming an opportunistic ant–plant interaction or mutualism (e.g., Heil and McKey 2003). Although recent monographic treatments explored the taxonomic utility of EFNs in *Senna* (Irwin and Barneby 1982; Randell 1988, 1989), little is known about their specific distribution, anatomy, and evolutionary significance in the genus.

Species of *Senna* were formerly included among the approximately 600 species of *Cassia* s.l. (Irwin and Turner 1960). Subsequent taxonomic treatments subdivided this large genus into the smaller *Cassia* s. str., *Chamaecrista* Moench, and *Senna*, and ascribed these three genera to subtribe Cassiinae (Irwin and Barneby 1981, 1982). In the most recent monograph, *Senna* comprised approximately 260 species (Irwin and Barneby 1982), which later increased to 350, mainly as a result of new nomenclatural combinations in non-American taxa (Randell and Barlow 1998). The separation of *Senna* from *Cassia* was confirmed by further taxonomic (e.g., Randell 1988, 1989, 1990; Singh 2001), structural (Endress 1994; Tucker 1996b), and phenetic studies (Boonkerd et al. 2005). Published molecular phylogenies, comprising only 11 species of *Senna*, supported its monophyly (Bruneau et al. 2001; see also Herendeen et al. 2003, based on both molecular and morphological data). In these phylogenies, Cassiinae are included in a large clade formed by other caesalpinioids (Caesalpinieae and Cassieae, subtribe Ceratoniinae) and the Mimosoideae (see also Wojciechowski et al. 2004). Although the recent survey on legumes by

Lewis et al. (2005) considered Cassiinae monophyletic (as Cassieae s. str.), relationships among the three genera *Cassia*, *Chamaecrista*, and *Senna* remain unclear.

The most recent classification of *Senna* (Irwin and Barneby 1982) relied greatly on Bentham's (1871) taxonomic revision of *Cassia*, specifically of *Cassia* subgenus *Senna*. Although Irwin and Barneby (1982) focused only on the American species, which represent the majority of the genus, their classification was soon adopted in subsequent treatments of *Senna* in other continents (discussed next). Irwin and Barneby (1982) divided *Senna* into six sections: *Astroites* (1 species, abbreviated to "sp." from here on), *Chamaefistula* (c. 140 spp.), *Paradictyon* (1 sp.), *Peiranisia* (c. 55 spp.), *Psilorhegma* (c. 30 spp.), and *Senna* (c. 20 spp.). They also recognized 35 series, which increased to 38 with more recent revisions of the Australian (Randell 1988, 1989, 1990) and Indian species of *Senna* (Singh 2001; see Appendix 1). This recent classification of *Senna* emphasized the importance of floral morphology, while former classifications (with *Senna* included in *Cassia*) focused on fruit structure (e.g., Bentham 1871). At a lower taxonomic level, the occurrence, location, and form of the EFNs were used in both older and more recent classifications of *Senna* (or as *Cassia*) to characterize several series or groups of series (Bentham 1871; Irwin and Barneby 1982).

In their classification of *Senna*, Irwin and Barneby (1982) proposed several hypotheses of evolutionary trends in floral morphology, focusing on androecium, corolla, and floral architecture. The fertility of all 10 stamens was used to distinguish section *Psilorhegma* from all other sections, which have only seven or fewer fertile stamens and three adaxial staminodes. The kind of androecium typical of *Psilorhegma* was considered to represent the ancestral state, justifying the "basal" position of *Psilorhegma* within *Senna*. Conversely, the highly asymmetric condition of the androecium and corolla characteristic of flowers in *Peiranisia* was used to interpret *Peiranisia* as the most derived section, while the flowers of sections *Chamaefistula*, *Astroites*, *Senna*, and *Paradictyon* were considered to represent intermediate evolutionary stages (Irwin and Barneby 1982; see also fig. 3 A–C). The monotypic sections *Astroites* and *Paradictyon* consist of *Senna villosa* and *S. paradictyon*, respectively. Although *S. villosa* shares some floral features with sect. *Chamaefistula*, and *S. paradictyon* with sections *Senna* and *Peiranisia*, the two species were assigned each to its own section because some of their morphological features are found nowhere else in the genus (Irwin and Barneby 1982). These unique features include the lomentaceous pod and stellate hairs of *S. villosa* and the xylopodium and parallel sepal venation of *S. paradictyon*.

The diversity of floral symmetries and structures raises interesting questions on the evolution of floral morphology in *Senna*. Irwin and Barneby (1982) suggested that floral asymmetry evolved in relation to bee pollination in more than one evolutionary line within *Senna*, but did not test this hypothesis, nor did other researchers. The relationship between enantiostyly and pollination biology has been investigated only in a few species of *Senna* (e.g., Fontanelle 1979; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003). Other morphological studies, which focused on a few species, examined the specialization of the stigma (Owens and Lewis 1989; Dulberger et al. 1994), the structure of the poricidal stamens (e.g., Lassaigne 1979; Endress 1994; Tucker 1996a), and floral development (Tucker 1996b).

The presence of EFNs represents another fascinating evolutionary aspect of *Senna* morphology. Most taxonomic treatments of *Senna* considered EFNs as an "archaic feature," present in the "basal" sections *Psilorhegma* and *Chamaefistula*, but lost in more "advanced" lines, i.e., sections *Senna*, *Paradictyon* and part of *Peiranisia* (Irwin and Barneby 1982; Randell 1989). Although little is known about EFNs in *Senna*, experimental studies in other angiosperms, including many legume taxa, demonstrated that the mutualistic relationship between EFNs and the ants that feed on them protects the plant from herbivores, thus enhancing plant fitness (e.g., Koptur 1979; Barton 1986; Rutter and Rausher 2004). Nevertheless, the implications of these recent findings for the evolutionary history of *Senna* have not been addressed. An explicit phylogenetic framework within *Senna* would allow us to investigate the proposed ancestral nature

of the EFNs and the single vs. multiple origins of these structures within the genus, compare the sizes of sister clades formed by species with or without EFNs, and discuss the potential evolutionary role of EFNs in light of their value for plant defense.

The major aim of the present study is to generate a molecular phylogeny of the genus *Senna* to be compared with current classification systems (Irwin and Barneby 1982; Randell 1988, 1989, 1990; Singh 2001) and elucidate patterns of morphological evolution. More specifically, we address the following questions: (1) Are the sections *Chamaefistula*, *Peiranisia*, *Psilorhegma*, and *Senna*, as defined by Irwin and Barneby (1982), monophyletic? (2) Are the monotypic *Astroites* and *Paradictyon* embedded in other sections or do they represent isolated lineages? (3) Which morphological traits are congruent with the clades defined by the molecular phylogeny? (4) Does our molecular tree support the hypotheses of floral evolution proposed by Irwin and Barneby (1982)? (5) Did EFNs evolve once or multiple times? (6) Are the EFNs associated with species-richer clades?

MATERIAL AND METHODS

Taxonomic sampling

Of the 98 taxa (101 samples) considered in this study, 87 were ascribed to *Senna*, and the remainder to other caesalpinioids (discussed later). The 81 *Senna* species (approximately one fourth of the genus) represent all six sections and 24 of 38 recognized series (Irwin and Barneby 1982; Randell 1988, 1989, 1990; Singh 2001; Appendix 1). Eleven of the 14 series unrepresented in our study are monotypic, while each of the remaining three series includes up to three species. Most samples were field-collected in Argentina, Australia, Bolivia, Brazil, Mexico, Panama, Paraguay, South Africa, and the United States, while a few were received from European and Australian botanic gardens. The broad infraspecific morphological variation of *Senna acuruensis*, *S. multijuga*, and *S. hirsuta* recognized at the varietal rank (Irwin and Barneby 1982), prompted us to sample several accessions of these species. The 11 outgroups, selected on the basis of published phylogenies, comprise species of *Cassia*, *Caesalpinia*, *Chamaecrista*, *Delonix*, and *Gleditsia* (Bruneau et al. 2001; Kajita et al. 2001; collection and voucher information provided in the Appendix 2).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica gel dried leaves and petals and from seeds. Tissue samples were homogenized using a Retsch MM 2000 shaker (Retsch, Haan, Germany). DNA was extracted using the Dneasy Plant Mini Kit (Qiagen, Basel, Switzerland), the protocol by Eichenberger et al. (2000), and the Extract-N-Amp Seed PCR Kit (Sigma, St. Louis, Missouri, USA). Three chloroplast regions (*rpL*16 intron, *rpS*16 intron, and the *matK* gene) were sequenced for all taxa. Target regions were amplified by the polymerase chain reaction (PCR; Mullis and Faloona 1987) on a Biometra TGradient thermocycler or a Biometra T1 thermocycler (Biometra, Göttingen, Germany). We used primer F71 (which anneals at position 71 of the flanking 3′ exon; Jordan et al. 1996) and the internal primer R1516 (Baum et al. 1998) to amplify a partial sequence of the *rpL*16 intron; primers rpsF and rpsR2 (Oxelman et al. 1997) to amplify the complete *rps*16 intron, and primers matK3R and matK3F (Sang et al. 1998) to amplify the central portion of the *matK* gene. Annealing positions of the *matK* primers are described in Sang et al. (1998). Primer sequences are listed in Table 1. The thermal cycling program for the amplification (TCPA) of the *rpL*16 and *rpS*16 introns consisted of the following steps: premelting at 95°C/4 min followed by 35 cycles with melting at 95°C/30 s, annealing at 51,3°C/1 min, extension at 72°C/1 min 50 s (*rpL*16) and at 1 min 30 s (*rpS*16). The TCPA for the *matK* gene was: premelting at 95°C/4 min followed by 5 cycles with melting at 95°/30 s, annealing at 42°C/1 min, extension at 72°C/1 min 42 s, and then 30 cycles with annealing at 50°C. All reactions ended with a final extension at 72°C/10 min followed by a holding step at 4°C. PCR products were electrophoresed on 1%

agarose gels containing ethidium bromide and examined under UV light. Successfully amplified PCR products were usually purified with the QIAquick PCR purification Kit (Qiagen), although shrimp alkaline phosphatase and exonuclease I (USB Corp., Cleveland, Ohio, USA) or the precipitation protocol by Dunn and Blattner (1987) were also employed.

Cycle-sequencing reactions were performed using the same primers (see Table 1) and the Big Dye Terminator (Perkin-Elmer, Applied Biosystems, Applera Europe BV, Rotkreuz, Switzerland), or the DYEnamic Et-terminator Kit (Amersham, Buckinghamshire, England) for Brazilian samples. Three additional internal primers, rpL16Fa rpL16Fb, and rpL16 Ra (Table 1), were designed from the conserved sites of the aligned *rpL*16 sequences from our taxa. Cycle sequencing products obtained using the Big Dye Terminator were cleaned with Microspin G-50 (Amersham Pharmacia Biotech Europe, Dübendorf, Switzerland) using multiscreen plates to remove excess Big Dye Terminator before loading on the automated sequencer ABI PRISM 3100 Genetic Analyzer (Perkin-Elmer). The cycle-sequencing products obtained with the DYEnamic Et-terminator Kit were directly loaded on the automated sequencer. The software Sequencher (versions 3.1.1 and 4.2; Gene Codes 1998) was used to edit and assemble complementary strands. Base positions were individually checked for agreement between the complementary strands. Sequences were visually aligned in MacClade 4.0 (Maddison and Maddison 2000). Sequence accession numbers are provided in Appendix 2.

Phylogenetic reconstruction

Cladistic analyses were performed using the maximum parsimony (MP) optimization criterion of the software package PAUP*: phylogenetic analyses using parsimony (*and other methods), version 4.0b10 (Swofford 2002). Only informative characters were included, and all characters and character states were weighted equally (Fitch 1971). Insertion/deletions (indels) were directly included in the overall analysis of the aligned matrices. Missing parts of a few sequences were replaced by question marks (see details in the Results, Size and structure of the molecular data sets).

The individual data matrices for each of the three DNA regions and a combined data matrix were analyzed by employing a heuristic search strategy with 1000 random taxon-addition replicates, holding 10 trees at each step, tree-bisection-reconnection (TBR) branch swapping with STEEPEST DESCENT and MULTREES options in effect, and saving a maximum of 30 trees for each replicate. Branch support was estimated by bootstrap resampling (Felsenstein 1985) with 1000 replicates, holding 10 trees at each step, simple addition sequence, and TBR branch swapping with STEEPEST DESCENT and MULTREES options in effect, saving a maximum of 30 trees in each replicate. Branch lengths of the trees derived from the combined data set were calculated by including all characters.

Our outgroups comprise species of *Cassia*, *Caesalpinia*, *Chamaecrista*, *Delonix*, and *Gleditsia* (see Appendix 2). To investigate whether *Senna* is monophyletic, we initially rooted the tree with *Gleditsia* alone, because recent molecular phylogenies identified *Gleditsia* as sister to a large clade that included all our sampled genera (Bruneau et al. 2001; Wojciechowski et al. 2004). Because this analysis supported the monophyly of *Senna*, we proceeded to keep only *Senna* species in the ingroup for the final analysis. The 11 outgroups were allowed to be paraphyletic with respect to the ingroup.

RESULTS

Size and structure of the molecular data sets

The length of the aligned combined data set included 2909 nucleotide positions (ntps), 501 (17.2%) of which were potentially parsimony informative (see Table 2). The partial sequences of the *matK* gene (620 aligned ntps), provided the highest percentage of informative positions (19.3%), while the *rpL*16 (1209 aligned ntps) and *rpS*16 (1080 aligned ntps) intron sequences were slightly less informative, with 18.6% and 14.4% informative positions, respectively. Absolute sequence length of the *rpL*16 intron ranged from 797 ntps (*S.* indet. ser. *Subverrucosae*) to 962 ntps (*Gleditsia triacanthos*), sequences of the *rpS*16 intron ranged from 839 ntps (*S. paradictyon*) to 908 ntps (*S. obtusifolia*), whereas partial sequences of the *matK* gene included 620 ntps.

Despite repeated efforts to optimize PCR conditions, we were unable to generate *matK* sequences for *S. aversiflora*, *S. odorata*, and one of the two accessions of *S. acuruensis* var. *acuruensis* (accession LQ 9201; see Appendix 2), and *rpL16* intron sequences for *S. alata*, *S. venusta*, and *Caesalpinia gilliesii*. Three sequences in the *rpL16* intron aligned data set are incomplete: *S. artemisioides* (110 missing ntps at the 3′ end), *S. cardiosperma* (141 missing ntps at the 3′ end), and *S. oligoclada* (161 missing ntps at the 3′ end). Also two sequences in the *rpS16* intron aligned data set are incomplete, *Caesalpinia gilliesii* (107 missing ntps at the 5′ end and 32 ntps at the 3′ end) and *Gleditsia sinensis* (181 missing ntps at the 3′ end).

Phylogenetic reconstruction

Descriptive values for the trees resulting from the analyses of the separate and combined data sets are listed in Table 2. The strict consensus trees generated from each separate data set showed no major topological conflicts (see fig. 1). Because the few observed topological discrepancies involved clades with weak branch support (bootstrap support [BS] < 50%), we combined all matrices in a combined data set that was used for MP analysis (Brower 1996; Nixon and Carpenter 1996). Minor topological discrepancies involved members of clades III (which formed a clade only in the *rpL16* strict consensus tree), IV, and VII. The tree derived from the combined data set showed increased resolution and branch support (see next paragraph, and also figs. 1 and 2).

Analyses of the combined data matrix using *Gleditsia* alone as outgroup supported the monophyly of *Senna*. Therefore, the final analyses reported here included only species of *Senna* in the ingroup (see fig. 2). MP analyses of the combined data set resulted in 29582 trees of 1136 steps, consistency index [CI] of 0.562 and retention index [RI] of 0.840, excluding uninformative characters. When all characters were included, the trees had a length of 1578 steps, CI = 0.674, and RI = 0.840. One of the MP phylograms (including all characters) is shown in fig. 3. Combination of the data sets clearly increased resolution and branch support in the resulting tree. For example, in the *Senna* clade the number of branches with BS above 80% approximately doubled in the combined tree as compared with the trees from the separate data sets. In addition, the number of branches with 100% BS increased to 16 in the combined tree, while only three branches had 100% BS in the trees from the separate data sets. The strict consensus (fig. 2) of the MP trees derived from the combined matrix supported the sister relationship between *Cassia fistula* and the *Senna* clade (90% BS). Analysis of the combined matrix also increased the support for the monophyly of *Senna* (100% BS), in comparison with the lower support retrieved from the separate data sets (58–87% BS). Within *Senna*, four species of section *Chamaefistula* (clade I) form the sister clade to the rest of the genus, which comprises clades II to VII.

None of the three chloroplast regions was sufficiently variable to resolve relationships within *Senna* (see figs. 1 and 2). This result is consistent with the findings of numerous studies, which have demonstrated that best phylogenetic resolution is often achieved through a

combination of several cpDNA regions in the same data set (Mast et al. 2001; Schönenberger and Conti 2001; Simões et al. 2004; Shaw et al. 2005).

DISCUSSION

Comparison between the molecular phylogeny and Irwin and Barneby's classification

Parsimony analysis of the combined data matrix supports the monophyly of *Senna* as defined by Irwin and Barneby (1981, 1982; see fig. 2), thus confirming the results of previous molecular phylogenetic studies, which included only a few *Senna* species (Bruneau et al. 2001, see also Herendeen et al. 2003). Our limited taxon sampling outside of *Senna* does not allow us to make any conclusive remarks concerning the monophyly and relationships of Cassiinae, although our results favor the sister relationship between *Cassia* and *Senna* (Bruneau et al. 2001), rather than between *Chamaecrista* and *Senna* (e.g., Herendeen et al. 2003).

Sectional delimitations within *Senna*, as defined by Irwin and Barneby (1982), are largely incongruent with our tree (fig. 2). Only section *Psilorhegma* is supported as monophyletic (82% BS), whereas sections *Chamaefistula*, *Peiranisia*, and *Senna* are paraphyletic. The largest section, *Chamaefistula*, is splintered into several lineages over the entire phylogenetic tree. Section *Peiranisia* is divided into two clades, and *Senna chloroclada*, ascribed to *Peiranisia*, groups instead with other species of *Chamaefistula*. The monotypic *Astroites* (*S. villosa*) is embedded within a clade comprising species of *Chamaefistula*, while the monotypic *Paradictyon* (*S. paradictyon*) is embedded within sect. *Senna*. Thus, our phylogenetic results do not support the conclusion that these monotypic sections represent isolated lineages within *Senna* (Irwin and Barneby 1982).

Although most of Irwin and Barneby's (1982) sectional delimitations are in conflict with our molecular results, series circumscriptions are often congruent with the molecular tree topology, as in the case of series *Aphyllae*, *Bacillares*, *Basiglandulosae*, *Deserticolae*, *Galeottianae*, *Isandrae*, and *Laxiflorae* (72–100% BS; fig. 2).

Phylogenetic relationships within Senna

The discussion of the seven major clades supported by the combined molecular phylogeny (fig. 2) will focus on the morphological synapomorphies that are congruent with these clades, with special emphasis on floral architecture and symmetry, and EFNs.

Clade I—Clade I, including only species of section *Chamaefistula*, is sister to the rest of *Senna* (fig. 2). This clade contains species ascribed to the American series *Galeottianae* and *Sapindifoliae*, and to the Asian series *Floridae*. These three series comprise trees and treelets lacking EFNs (fig. 3). Their monosymmetric flowers have seven fertile stamens and three adaxial staminodes. This androecial organization is also characteristic of most remaining clades, except for IV and VII (see fig. 3A). In some flowers, the gynoecium may be slightly deflected to one side. Because carpel deflection is inconsistent in these species, their flowers are not considered truly enantiostylous.

Previous taxonomic treatments of *Senna* identified similarities in the habit, corolla, and androecium of the species included in clade I (Bentham 1871; Irwin and Barneby 1982). However, these species were ascribed to different series in section *Chamaefistula* mainly based on allopatric ranges of distribution and morphological differences among their hypanthia and fruits (Irwin and Barneby 1982).

Clade II—Clade II is formed by Australian, American, and African species of section *Senna*, except for the South American *S. paradictyon*, the only species ascribed to section *Paradictyon* (fig. 2). Therefore, our results suggest that sect. *Senna* would be monophyletic, if *S. paradictyon* were included in it. The clade comprises shrubs and treelets characterized by asymmetric flowers and lacking EFNs (fig. 3). Floral asymmetry appears to be a synapomorphy for the super clade comprising clades II–VII, with a possible reversal to monosymmetric flowers in clade VII. In clade II, floral asymmetry affects only the gynoecium, while the androecium and corolla are monosymmetric. These flowers represent the simplest variant of enantiostyly, involving the lateral deflection of the gynoecium only (see fig. 3B). The petals partially enclose the reproductive organs during the initial stages of anthesis and unfold during the later stages, as in *S. didymobotrya* (Dulberger 1981; B. Marazzi, personal observation) or never unfold, as in *S. alata*. The orientation of the two lateral abaxial stamens is characteristic for this clade and has been used to distinguish sect. *Senna* from sect. *Chamaefistula* (Irwin and Barneby 1982; see also figs. 3A, B). Of the three abaxial stamens, one is median and two are lateral; these latter ones are curved laterally, so that their anthers face each other, resembling the arms of tongs.

The presence of a xylopodium (discussed later), bracteoles on the pedicels (otherwise absent in *Senna*), and parallel venation of the sepals (otherwise palmately veined in *Senna*) are autapomorphies of *S. paradictyon* and prompted its inclusion in a separate, monotypic section (Irwin and Barneby 1982). In contrast, Bentham (1871) included *S. paradictyon* (as *Cassia paradictyon*) in his section *Chamaesenna* ser. *Pictae*, which later became ser. *Pictae* of section *Senna* in Irwin and Barneby's (1982) revision, although *S. paradictyon* was excluded from it. The xylopodium, a cylindrical, lignified region of the stem that is located just above the soil level, gives rise to new sprouts. This interesting structure of *S. paradictyon* may represent an adaptation to fire, which plays an important ecological role in the renewal of cerrado vegetation (Ratter et al. 1997), where *S. paradictyon* typically occurs. The cerrado is a vegetation type of central Brazil, extending marginally into Paraguay and Bolivia, characterized by savanna woodlands (e.g., Ratter et al. 1997; Pennington et al. 2000).

Clade III—Clade III is formed by a part of the polyphyletic section *Peiranisia* and combines the southern North American and northern Central American series *Deserticolae* and *Isandrae* and the monotypic South American series *Excelsae*. All species of this clade are treelets and shrubs lacking EFNs and characterized by enantiostylous flowers, in which the floral asymmetry also involves the corolla and, in some species, the androecium (fig. 3). Enantiostyly affects petals and stamens in different ways, for example: (1) the upper petals are more or less reduced, (2) one or both lower petals are strongly modified in shape and size, (3) the abaxial stamens are on the opposite side of the deflected carpel, or (4) the androecium is nearly monosymmetric. Therefore, many variants of enantiostyly are represented in the clade. The observation that floral asymmetry assumes different variants in this and other clades (discussed later) and that it was derived independently multiple times in *Senna* suggests that such variants probably originated through different evolutionary and developmental pathways, therefore they are not strictly homologous.

The highly asymmetric corolla, in which one or both lower petals are strongly modified in shape and size, was used to distinguish section *Peiranisia* from sections *Chamaefistula* and *Senna* (Irwin and Barneby 1982; see also figs. 3A-C). Our results divide *Peiranisia* into two well-supported clades (III and VI; BS = 84% and 99%, respectively), except for *S. chloroclada* of the monotypic series *Chlorocladae*, which is included in clade IV. Irwin and Barneby's (1982) classification used androecial traits and the absence of EFNs to distinguish the series of *Peiranisia* in clade III from the other series of the section, i.e., series *Chlorocladae* in clade IV, and *Interglandulosae* in clade VI (fig. 2). In addition, other characteristics of the androecium, such as minor differences in the size and form of the stamens, were used to distinguish the series in clade III from each other (Irwin and Barneby 1982).

Clade IV—In clade IV, one species of section *Chamaefistula*, *S. skinneri*, is sister to a clade formed by subclades IVa and IVb. Subclade IVa comprises only members of section *Psilorhegma*, which is thus supported as monophyletic by our analyses. In subclade IVb, one species of section *Peiranisia*, *S. chloroclada*, is sister to a clade comprising species of *Chamaefistula*, series *Bacillares* and *Trigonelloideae* (subclade IVb; fig. 2). All species in subclade IVa (sect. *Psilorhegma*) occur exclusively in Australia, while the other members of clade IV are American. Clade IV includes herbs, shrubs, and treelets with asymmetric flowers and bearing EFNs on the leaves (fig. 3). Floral asymmetry involves corolla and androecium, in addition to the gynoecium. One or both lower petals are often strongly concave, and, in a few species, they surround the reproductive organs and can be additionally modified in shape and size (e.g., *S. chloroclada*). In some species, the flowers are slightly rotated on their axis and attain an oblique position (e.g., *S. aciphylla*, *S. obtusifolia*), further deflecting the gynoecium, thus emphasizing enantiostyly. Hairs were observed on the anthers of both middle and abaxial stamens in species of clade IV, but not of other clades. While hairs are normally rare on the anthers of caesalpinioids (Endress and Stumpf 1991; Tucker 1996a), in *Senna* they may represent a synapomorphy for clade IV.

The fertility of all 10 stamens in the androecium, traditionally used to circumscribe section *Psilorhegma* (Bentham, 1871, as *Cassia*; Irwin and Barneby 1982), represents a unique synapomorphy for subclade IVa (see figs. 2 and 3). In section *Psilorhegma*, the stamens are more or less all similar in shape, but in some species (e.g., *S. artemisioides*) the lateral abaxial stamen opposite to the deflected carpel is bigger. In the remaining species of clade IV (with seven or rarely fewer fertile stamens), the abaxial stamens present two kinds of arrangement: either they are almost equal in size to the middle stamens, and, in this case, the androecium is nearly monosymmetric, or they are much longer, and, in this case, the median abaxial stamen is deflected to the opposite side of the gynoecium, making the androecium asymmetric.

The 11 sampled species of series *Bacillares* (sect. *Chamaefistula*) are all included in subclade IVb (fig. 2). Series *Bacillares*, comprising approximately 50 species of shrubs and treelets, is characterized by leaves consistently having two pairs of leaflets (Irwin and Barneby 1982). Members occur in tropical and subtropical areas of the American continent and have colonized a wide range of habitats, from the dry cerrado to the humid tropical forest. Some species, e.g., *S. macranthera*, display a high level of morphological variation, which prompted the description of many infra-specific varieties (Irwin and Barneby 1982). Interestingly, Irwin and Barneby (1982) did not consider the flowers of *Bacillares* as enantiostylous, but rather as having a "centrically (or subcentrically) oriented pistil," and interpreted the asymmetric corolla and the median pistil as a "variation of simple zygomorphy." Flowers of one species, *S. hayesiana*, lack the adaxial staminodes and the abaxial stamens, displaying only the four middle stamens (Irwin and Barneby 1982; Marazzi, personal observation; fig. 3). Our molecular phylogeny corroborates the monophyly of *Bacillares* and its subdivision into two well-supported subclades (BS values of 97% and 84%; fig. 2): one subclade consists of a large polytomy with short terminal branches, suggesting a possibly recent radiation of this group; the other subclade comprises only a few species at the tips of longer terminal branches (*S. hayesiana*, *S. herzogii*, and *S.* indet. ser. *Bacillares*; fig. 3). We were unable to identify any morphological or biogeographic patterns congruent with these subclades.

In our chloroplast DNA phylogeny, *S. chloroclada*, traditionally ascribed to section *Peiranisia*, appears to be more closely related to species of *Chamaefistula* in clade IV (80% BS) than to species of *Peiranisia* (fig. 2). This subaphyllous species was viewed as representing a transitional stage between the aphyllous and the leafy species (Burkart 1943). Irwin and Barneby (1982) placed *S. chloroclada* in *Peiranisia*, ser. *Chlorocladae* for its habit, inflorescence, asymmetric corolla, and pod similar to that of *Peiranisia*, ser. *Aphyllae*. However, they also mentioned that the anthers of *S. chloroclada* resemble those of *S. mucronifera*, assigned to sect. *Chamaefistula*. Our results suggest that the morphological similarities between *S. chloroclada* and section *Peiranisia*, ser. *Aphyllae* have independent evolutionary origins. The observation that *S.*

chloroclada is associated with a rather long branch (29 nucleotide substitutions in one of the MP trees; see fig. 3), the unusual combination of traits similar to those found in members of disparate clades (clades III, IV, and VI), and its distribution restricted to the Gran Chaco (including Argentina, Bolivia and Paraguay) justify its isolated taxonomic position.

Senna skinneri, ascribed to the monotypic series *Skinnerae* (sect. *Chamaefistula*), is sister to the remainder of clade IV. This species shares some morphological traits with species of both subclades IVa and IVb. Its flowers resemble those of many species of subclade IVb, but the leaves are more similar to those of subclade IVa in having more than three pairs of leaflets. Irwin and Barneby (1982) suggested that *S. skinneri* had an unspecified "close resemblance" and "genetic affinity" to series *Coriaceae* and *Laxiflorae* or was related to ser. *Spinescentes* based on similarities in pod morphology (the three series belonging to section *Chamaefistula*). Series *Coriaceae* and *Spinescentes* are not represented in our molecular phylogenetic study (see Appendix 1), and series *Laxiflorae* is included in clade V (fig. 2). Therefore, the inclusion of representatives from series *Coriaceae* and *Spinescentes* will be necessary to clarify the phylogenetic position of *S. skinneri* in the genus.

Clade V—The strongly supported clade V (100% BS) contains the monotypic series *Confertae* (*S. uniflora*) and members of series *Laxiflorae*, both of section *Chamaefistula* (fig. 2). The two series are strikingly different in habit and floral structure. *Senna uniflora* is a widespread, weedy, self-pollinated herb with small enantiostylous flowers and a monosymmetric corolla. Conversely, *Laxiflorae* comprise shrubs or treelets restricted to Brazil (except for one species extending into Bolivia and Paraguay), with rather showy enantiostylous flowers and a slightly asymmetric corolla characterized by one lower petal slightly modified in form and more concave than the other lower petal (Irwin and Barneby 1982). The unusual angular shape and marginal teeth of the leaflets typical of *S. phlebadenia* (series *Laxiflorae*) form a combination of traits unique in *Senna* and indeed in Cassiinae (Irwin and Barneby 1985). A synapomorphic feature for clade V is the presence of EFNs at the base of the pedicels (fig. 3), in addition to the occurrence of similar nectaries on the leaves. Because pedicellar nectaries are restricted to *S. uniflora* and series *Laxiflorae*, Irwin and Barneby (1982) suggested that *S. uniflora* was a "specialized offshoot" derived from series *Laxiflorae* or from the common ancestor of *S. uniflora* and series *Laxiflorae*. The molecular tree supports this latter conclusion.

Series *Laxiflorae* seems to be still poorly known, and its species number has increased in the past decades: *S. phlebadenia* (Irwin and Barneby 1985) was added to the four traditionally recognized species (*S. australis*, *S. cana*, *S. lechriosperma*, and *S. velutina*; Irwin and Barneby 1982), and recently, a new species was discovered (A. Araujo and V. C. Souza, Departamento de Ciências Biológicas, University of São Paulo, Brazil, unpublished data). In addition, species delimitation appears to be problematic in this series because flowers of *Laxiflorae* are morphologically similar, and fruit characteristics are needed for species identification (Irwin and Barneby 1982). The inclusion of more taxa ascribed to *Laxiflorae* would allow us to further test the sister relationship between *S. uniflora* and *Laxiflorae* and to further explore species boundaries in *Laxiflorae*.

Clade VI—Clade VI comprises series *Aphyllae* and *Interglandulosae* of section *Peiranisia*, with *Aphyllae* forming a strongly supported (100% BS) monophyletic group nested within *Interglandulosae*. This exclusively American clade comprises species of shrubs and treelets bearing EFNs on the leaves and strongly asymmetric flowers (figs. 2 and 3). Floral asymmetry affects the corolla and the androecium, in addition to the gynoecium (see fig. 3C). Both lower petals are strongly modified in shape and size, and in most species one or both are incurved in front of the reproductive structures, hiding all or part of them. These petals were suggested to function as an "androecial shield" (Irwin and Barneby 1982), but this proposed function has not

yet been convincingly demonstrated. The upper petals may be more or less reduced. The abaxial stamens are often all deflected to the opposite side of the gynoecium.

The shrub, treelet, or tree species of section *Peiranisia* series *Interglandulosae* occur mainly in more or less wet habitats (usually with a pronounced dry season), ranging from northeastern Brazil and northern South America to Central America and Mexico (Irwin and Barneby 1982). In many species, the leaves have up to more than 14 leaflet pairs and, in others, the upper petals are more or less reduced, while the anthers of the abaxial stamens have peculiar long beaks. In contrast to the distribution of *Interglandulosae*, members of *Aphyllae* occur in dry areas of northern and northwestern Argentina, southeastern Bolivia, and adjacent northwestern Paraguay (Irwin and Barneby 1982). These species are highly xerophytic shrubs with usually aphyllous and photosynthetic stems, a combination of features unique among *Senna* species. Leaves are only present in young shoots (Bravo 1978; Irwin and Barneby 1982; Marazzi, personal observation). The scales present on the stems have been interpreted as reduced leaves (Bentham, 1871; Burkart 1943; Bravo 1978) and stipules (Irwin and Barneby 1982). The upper petals are not reduced, and the anthers of the abaxial stamens appear beakless.

The samples of *S. acuruensis* (restricted to northeastern Brazil) and *S. multijuga* (ranging from northeastern Brazil to northern South America and Mexico) form a strongly supported clade (97% BS; fig. 2). *Senna acuruensis* is paraphyletic in the cpDNA phylogeny. The broad range of morphological variation characterizing *S. acuruensis* and *S. multijuga* prompted the recognition of several infraspecific varieties (Irwin and Barneby 1982). These two species are distinguished mainly by the habit (large trees in *S. multijuga* vs. spindly shrubs in *S. acuruensis*) and by the presence of viscid hairs on the axis of the inflorescence in *S. acuruensis.* Variation in the number of leaflet pairs has been used to recognize three varieties in *S. acuruensis*: plants with more than 14 pairs of leaflets (multifoliolate) have been assigned to var. *acuruensis*, while plants with less than 14 pairs of leaflets (few-foliolate) have been assigned to var. *catingae* and var. *interjecta*. In *S. multijuga*, all varieties have more than 14 pairs of leaflets. Irwin and Barneby (1982) proposed that *S. acuruensis* is the closest relative of *S. multijuga* and that this species pair is closely related to the other species of the same series (*S. aristeguietae* and *S. trachypus*, both few-foliolate) and to *S. mutisiana, S. williamsii*, and one form of *S. pallida* (all three taxa are multifoliolate). Our phylogenetic analyses support the monophyly of a multifoliolate group and a few-foliolate group, suggesting that the two sampled varieties of *S. acuruensis* may in fact represent different species (fig. 2). However, because our sampling included only two out of three and two out of five described varieties in *S. acuruensis* and *S. multijuga*, respectively, it is premature to draw any final conclusions on species circumscriptions in this complex.

Clade VII—Clade VII comprises the monotypic section *Astroites* (*S. villosa*) and species of section *Chamaefistula*. Most species are American and one, *S. barclayana*, is Australian (Randell 1988; fig. 2). Members of clade VII are mainly herbs and shrubs, sometimes weeds, with monosymmetric flowers and bearing one or more EFNs on the leaves (fig. 3). The clade is divided into two strongly supported subclades (VIIa, 94% BS, and VIIb, 100% BS; fig. 2).

Subclade VIIa includes *S. purpusii* (ser. *Pachycarpae*), a group of species mainly of ser. *Coluteoideae* (with *S. birostris* of ser. *Stipulaceae* nested within) and the monophyletic ser. *Basiglandulosae* (all of section *Chamaefistula*). Their flowers have sometimes six, instead of seven, fertile stamens, because the median abaxial stamen is highly reduced and sterile or absent (Irwin and Barneby 1982). In some species, the gynoecium may be slightly deflected to the right or to the left, but such flowers are not considered truly enantiostylous because they occur in inflorescences formed also by exactly monosymmetric flowers (see discussion of clade I). *Senna hirsuta* is paraphyletic in our cpDNA phylogeny (fig. 2). This species comprises many varieties (Irwin and Barneby 1982), which previous taxonomic studies considered to represent different species (as belonging to *Cassia*; e.g., Bentham, 1871). In our phylogenetic tree, series *Stipulaceae*, represented only by *S. birostris*, is embedded into series *Coluteoideae* (fig. 2). Irwin

and Barneby (1982) created *Stipulaceae* to accommodate those species of section *Chamaefistula* displaying a mixture of seed and fruit characteristics typical of series *Coluteoideae* and *Pachycarpae* (Irwin and Barneby 1982). These species were previously included in *Cassia* ser. *Pachycarpae*, which was therefore "diagnostically inseparable" from *Coluteoideae* (Bentham, 1871). Expanded taxon sampling in series *Pachycarpae* and *Stipulaceae* is necessary to clarify the relationships among series *Coluteoideae, Pachycarpae,* and *Stipulaceae*.

Subclade VIIb comprises the monotypic section *Astroites* (*S. villosa)*, the monotypic series *Armatae* (*S. armata*) of section *Chamaefistula*, and species of sect. *Chamaefistula* ser. *Brachycarpae*. Irwin and Barneby (1982) noticed similarities between the flowers of *S. armata* and *S. villosa* and those of ser. *Brachycarpae*, but placed *S. armata* in its own series, *Armatae*, because of its xeromorphic habit, and *S. villosa* in its own section, *Astroites,* because of its lomentaceous pod and uncommon stellate hairs. In contrast, Bentham (1871) grouped *S. villosa* (as *Cassia villosa*) together with *S. uniflora* (as *C. sericea*) of clade V.

The most evident morphological difference between subclades VIIa and VIIb concerns the androecium. In the first subclade, the six to seven fertile stamens are differentiated into a middle and an abaxial set, with the four middle stamens notably shorter than the abaxial ones. In contrast, in the second subclade, all fertile stamens have more or less the same length and shape.

Evolutionary aspects of floral morphology

The current classification of *Senna* reflects Irwin and Barneby's (1982) interpretation of evolutionary trends in the floral morphology of the genus, focusing in particular on androecium, corolla, and floral architecture. In their view, fertility of all 10 stamens represented the ancestral condition of the androecium in *Senna*, and they assigned all species with this trait to sect. *Psilorhegma*. All remaining sections were characterized by the supposedly derived state with seven or fewer fertile stamens. Within these sections, the highly asymmetric flowers of *Peiranisia* were considered the most derived state of floral architecture, in contrast with the ancestral, monosymmetric flowers of *Chamaefistula* (Irwin and Barneby 1982; see also Randell 1989, for a detailed list of "primitive" vs. "advanced" character states). However, Irwin and Barneby (1982) did acknowledge the difficulty of identifying a clear evolutionary progression in the floral morphology of extant species of *Senna* because the presence of strongly specialized features, either in the corolla or in the androecium, produced a mosaic of ancestral and derived states in any given species that hampered the recognition of unequivocal synapomorphies. The cpDNA phylogeny presented here suggests that the morphological features used by Irwin and Barneby (1982) for their classification evolved independently multiple times, thus limiting their taxonomic utility.

For example, the position of section *Psilorhegma,* embedded in clade IV, does not support Irwin and Barneby's (1982) conclusion that the fertility of all ten stamens represents the ancestral condition of the androecium in *Senna*, but rather a synapomorphy of a well supported subgroup of *Senna* (see discussion of clade IV; fig. 2). The observation that the androecium consists of seven or fewer fertile stamens and three staminodes in all sections of *Senna*, except for *Psilorhegma,* suggests that this androecial organization probably characterized the ancestral *Senna* flower.

Different patterns of floral symmetry characterize different clades (or subclades) of the molecular phylogeny presented in this study. The species with monosymmetric flowers group into two clades (I and VII), whereas the species with asymmetric flowers form the remaining clades II to VI (fig. 3). Because flowers in *Cassia* s.str., the putative sister of *Senna* (this study and Bruneau et al. 2001), are monosymmetric (Irwin and Barneby 1982), the flowers of *Senna* were probably ancestrally monosymmetric, with a later switch to asymmetry. Therefore, the monosymmetry of flowers in clade VII probably represents a reversal to the ancestral condition. The polyphyly of section *Peiranisia,* split over three different clades, suggests that strong floral asymmetry, affecting corolla, androecium, and gynoecium, evolved independently more than once (see fig. 2).

Floral asymmetry in *Senna* affects gynoecium, androecium, and corolla, but the three whorls do not always simultaneously contribute to the asymmetry. The gynoecium alone, both gynoecium and corolla, or gynoecium, corolla and also androecium can be involved, as in clades II, III, and III–VI, respectively. Clade III is characterized by several different variants of floral asymmetry. Because all asymmetric flowers observed in this study are enantiostylous, we doubt the existence in ser. *Bacillares* of non-enantiostylous flowers with the floral asymmetry affecting only the corolla, as reported by Irwin and Barneby (1982). The existence of different variants of asymmetric flowers and the independent switches to these variants inferred from the molecular phylogeny suggest that our traditional interpretation of floral asymmetry in *Senna* may be inadequate, for it subsumes different morphological, evolutionary, and, probably, developmental pathways. In upcoming structural and developmental studies, we will investigate in depth the diversity of asymmetric patterns in *Senna* flowers and with future optimization analyses will evaluate possible scenarios for the evolution of diverse types of floral symmetry.

Evolutionary role of extrafloral nectaries

Many species of *Senna* bear EFNs on the leaves, and a few species also bear them on the pedicels. The great variation in the shape, number, and location of the EFNs has been used for series circumscription in several *Senna* classifications (e.g., as *Cassia* in Bentham, 1871; Irwin and Barneby 1982). Recent monographic treatments viewed the presence of EFNs as an "archaic feature," for EFNs are present in the supposedly "basal" section *Psilorhegma*, but absent in the more "advanced" sections *Senna*, *Paradictyon*, and part of *Peiranisia* (Irwin and Barneby 1982; Randell 1989, 1990). However, our phylogenetic results reject this interpretation as all sampled species with EFNs form a strongly supported clade, the "EFN clade" (93% BS; figs. 2 and 3), embedded within *Senna*. Therefore, the presence of EFNs constitutes a clear synapomorphy for the four clades IV–VII (fig. 3). EFNs appear to have evolved once in *Senna*, being later lost or suppressed in the highly xerophytic series *Aphyllae*, where they have never been observed. The presumed absence of EFNs in *Aphyllae* (Irwin and Barneby 1981) may be related to the absence of normally developed leaves in the adult plants (see also the discussion of clade VI). However, EFNs may be present in young shoots of *Aphyllae* where leaves are produced, but the developmental morphology of young shoots in this series has not yet been investigated.

The distribution of EFNs is associated with strong clade-size disparity (fig. 3). The difference in clade size is unlikely to be an artifact of taxon sampling because we sampled all sections and series according to species number (see Appendix 1, Appendix 2). The EFN clade, including 61 species, is sister to the EFN-less clade III, which includes only six species (fig. 3). This disparity corresponds to a 10-fold difference in clade size. Because sister clades are the products of the same cladogenetic event and, by definition, of equal age (Hennig 1966), the observed disparity in species numbers could reflect different rates of speciation and/or extinction within each clade. Molecular dating analyses to evaluate the age of the respective crown groups will be necessary to clarify the link between diversification rates and clade size disparity. Interestingly, species with EFNs and species without EFNs occur together in some continents, i.e., America (most species) and Australia (especially subclade IVa; fig. 2). However, species included in the EFN clade have colonized a wider range of habitats and climates, including rain forests, savannas, cerrados, and deserts, and display a higher diversity of habits, including trees, treelets, shrubs, lianas, and herbs (often ruderals or weeds), than species lacking EFNs. The ant–plant protective mutualism and its likely positive effect on plant fitness might explain, at least in part, the higher species richness of the EFN clade in *Senna*, and the greater diversity of habitat and habits observed for the clade.

Plants with EFNs offer nectar to ants, which, in return, protect the plant from herbivores and seed predators (Tilman 1978; Smiley 1986). The documented effects of such protection on plant fitness include decreased damage due to herbivory (Stephenson 1982; Koptur et al. 1998, Rutter and Rausher 2004), increased plant growth and survival (Buckley 1983; Barton 1986;

Kelly 1986), and higher seed set (Koptur 1979; Barton 1986; Oliveira 1997). Ants were observed to feed on the EFNs of *Senna* species (e.g., Schupp and Feener 1991; Marazzi, personal observation). Exclosure experiments are in progress to test whether ants visiting EFNs contribute to decreased herbivory and higher seed production in *S. mexicana* (Koptur 2004). Many genera in Leguminosae have EFNs, but few researchers have investigated the interaction with ants in any detail (see McKey 1989, for a review). For example, studies on *Acacia* provided the first convincing evidence for the protective function of the mutualism (Janzen 1967). Other studies have shown the mutualistic relationship with ants in *Inga* (Koptur 1984, 1994; Wickers 1993) and in *Chamaecrista* (Barton 1986; Kelly 1986; Rutter and Rausher 2004), confirming the positive effects of the mutualism on plant fitness.

The mutualism in *Senna* has not yet been experimentally investigated. However, because the morphology of the EFNs in *Senna* and *Chamaecrista* is similar (Pascal et al. 2000), we expect the EFNs in *Senna* to play a protective role similar to that documented in *Chamaecrista* and other legume taxa. Therefore, it is probable that the evolution of EFNs conferred higher fitness to the EFN clade, increasing survival, reproduction, dispersal, and potential for adaptation. Consequently, the evolution of EFNs might have played a key role in producing a higher diversification rate, thus explaining, at least in part, the observed size difference between the EFN clade and the EFN-less clades, in particular clade III (figs. 2 and 3). To summarize, the available phylogenetic and ecological evidence suggests that EFNs in *Senna* may represent a key innovation in plant defense strategies that promoted large-scale diversification and colonization of a wide range of habitats and climates in different continents, especially in America and Australia.

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TABLE 1

Primer sequences for the cpDNA regions used in this study.

Primer	5′ to 3′
rpL16 intron: F71	GCTATGCTTAGTGTGTGACTCGTTG
R1516	CCCTTCATTCTTCCTCTATGTTG
rpL16Fa	ATCTCTACTACAGAACCG
rpL16Fb	TTTKGGGTTATAGTTGATG
rpL16Ra	CTATARAACTAATAACCAAC
rpS16 intron: rpsF	GTGGTAGAAAGCAACGTGCGACTT
rps2R	TCGGGATCGAACATCAATTGCAAC
matK gene:	
matK3F	AAGATGCCTCTTCTTTGCAT
matK3R	GATCCGCTGTGATAATGAGA

Note: PrimerS rpL16Fa, rpL16Fb, and rpL16Ra were used exclusively for cycle sequencing.

TABLE 2

Description of cpDNA data sets and resulting trees (excluding uninformative characters).

cpDNA region/matrix	Aligned length	Informative ntps (% of aligned ntps)	No. of steps	CI	RI	No. of trees
rpL16 intron	1209	225 (18.6)	511	0.568	0.849	13530
rpS16 intron	1080	165 (14.4)	335	0.570	0.856	18150
matK gene (part)	620	120 (19.4)	262	0.599	0.840	2917
combined	2909	501 (17.2)	1136	0.562	0.840	29582

Note: ntps = nucleotide positions, CI = consistency index, RI= retention index.

APPENDIX 1

Classification of *Senna* used in the present study based on Irwin and Barneby (1982), Randell (1988 1989, 1990), and Singh (2001). The order of the sections is after Irwin and Barneby (1982), while series are listed alphabetically. Asterisks indicate monotypic sections or series. Rectangular brackets include abbreviations of sections and series listed in fig. 2 and in Appendix 2; series not represented in this study are labeled with [n.r.].

Section *Psilorhegma* (Vogel) H.S. Irwin & Barneby [PS]

- Series *Davidsonae** V. Singh [n.r.]
- Series *Interglandulosae* (Benth.) Randell [Int]
- Series *Oligocladae* Randell [Oli]
- Series *Subverrucosae* (Benth.) Randell [Sub]
- Series *Sulfureae** V. Singh [n.r.]

Section *Chamaefistula* (Collad.) H.S. Irwin & Barneby

- Series *Armatae** H.S. Irwin & Barneby [Arm]
- Series *Bacillares* (Benth.) H.S. Irwin & Barneby [Bac]
- Series *Basiglandulosae* (Collad.) H.S. Irwin & Barneby [Bas]
- Series *Brachycarpae* (Benth.) H.S. Irwin & Barneby [Bra]
- Series *Coluteoideae* (Collad.) H.S. Irwin & Barneby [Col]
- Series *Confertae** (Benth.) H.S. Irwin & Barneby [Con]
- Series *Coriaceae* (Benth.) H.S. Irwin & Barneby [n.r.]
- Series *Floridae* (Benth.) H.S. Irwin & Barneby [Flo]
- Series *Galeottianae* H.S. Irwin & Barneby [Gal]
- Series *Harleyanae** H.S. Irwin & Barneby [n.r.]
- Series *Laxiflorae* (Benth.) H.S. Irwin & Barneby [Lax]
- Series *Nanae** H.S. Irwin & Barneby [n.r.]
- Series *Pachycarpae* (Benth.) H.S. Irwin & Barneby [Pac]
- Series *Sapidifoliae* H.S. Irwin & Barneby [Sap]
- Series *Skinneranae** H.S. Irwin & Barneby [Ski]
- Series *Spinescentes* H.S. Irwin & Barneby [n.r.]
- Series *Stipulaceae* H.S. Irwin & Barneby [Sti]
- Series *Temperatae* H.S. Irwin & Barneby [n.r.]
- Series *Tharpia** (Britton & Rose) H.S. Irwin & Barneby [n.r.]
- Series *Trigonelloideae* (Collad.) H.S. Irwin & Barneby [Tri]
- Series *Trolliflorae** H.S. Irwin & Barneby [n.r.]

Section *Astroites H.S. Irwin & Barneby [AS]**

Section *Senna* Mill. [SE]

- Series *Aculeatae** H.S. Irwin & Barneby [n.r.]
- Series *Pictae* (Benth.) H.S. Irwin & Barneby [Pic]
- Series *Senna* Mill. [Sen]
- Series *Spinigerae** H.S. Irwin & Barneby [n.r.]

Section *Paradictyon H.S. Irwin & Barneby [PA]**

Section *Peiranisia* (Raf.) H.S. Irwin & Barneby [PE]

- Series *Aphyllae* (Benth.) H.S. Irwin & Barneby [Aph]
- Series *Auriculatae** (Benth.) V. Singh [n.r.]
- Series *Chlorocladae** H.S. Irwin & Barneby [Chl]
- Series *Deserticolae* H.S. Irwin & Barneby [Des]
- Series *Egregiae** H.S. Irwin & Barneby [n.r.]
- Series *Excelsae** (Benth.) H.S. Irwin & Barneby [Exc]
- Series *Interglandulosae* (Benth.) H.S. Irwin & Barneby [Itg]
- Series *Isandrae* H.S. Irwin & Barneby [Isa]

APPENDIX 2

Taxa used in this study, GenBank accession numbers for the three chloroplast regions studied, source, and voucher information. A dash indicates the region was not sampled. See Appendix 1 for full section and series names of *Senna* species. Voucher specimens are deposited in the following herbaria (in alphabetic order): ANBG (= CBG) = Australian National Botanic Gardens, BGB = Botanical Garden of the University of Basel, BGM = Botanischer Garten der Universität München, CTES = Instituto de Botánica del Nordeste, Corrientes, HUEFS = Universidad Estadual de Feira de Santana, KPBG = Kings Park and Botanic Garden, Perth, LPB = Herbario Nacional de Bolivia, La Paz, MEXU = Universidad Nacional Autónoma de México, MT = Université de Montréal, NMC = New Mexico State University, Las Cruces, PBIB = Parco Botanico Isole di Brissago, PERTH = Department of Conservation and Land Management, Perth, PMA = Universidad de Panamá, PY = Museo Nacional de Historia Natural de Paraguay, RBGA = Royal Botanic Garden Mount Annan, SI = Instituto de Botánica Darwinion, San Isidro, STRI = Smithsonian Tropical Research Institute, Balboa, SYD = University of Sydney, Z = University of Zürich and Botanical Garden.

Taxon; Section, Series [given only for *Senna* species]; *rpL*16, *rpS*16, *matK*; Source; Voucher specimen.

Caesalpinia decapetala (Roth) Alston; AM086721; AM086910; AM086828; Cult. PBIB 2003/77; *Marazzi BM137*, garden, Z. ***C. gilliesii*** (Hook.) Benth.; —, AM086914, AM086829, Wild; *Marazzi et al. BM131*, Argentina, Tucuman, CTES, Z.

Cassia fistula L.; AM086721, AM086915, AM086830; Cult. at the roadside; *Marazzi & Flores BM177*, Mexico, Oaxaca, MEXU, Z.

Chamaecrista acosmifolia (Benth.) H.S. Irwin & Barneby; AM086567, AM086584, AM086602; Wild; *Conceiçao & Marazzi AC1129*, Brazil, Bahia, HUEFS, Z. ***C. desvauxii*** (Collad.) Killip; AM086715, AM086911, AM086831; Wild; *Marazzi et al. BM013*, Paraguay, San Pedro, PY, CTES, Z. ***C. eitenorum* var. *regana*** (H.S. Irwin & Barneby) H.S. Irwin & Barneby; AM086566, AM086585, AM086603; Wild; *Conceiçao & Marazzi AC1133*, Brazil, Bahia, HUEFS, Z. ***C. nictitans*** Moench; AM086721, AM086912, AM086832; Wild; *Marazzi et al. BM034*, Paraguay, Alto Paraná, PY, CTES, Z. ***C. serpens*** Greene; AM086717, AM086913, AM086833; Wild; *Marazzi & Flores BM179*, Mexico, Oaxaca, MEXU, Z.

Delonix regia (Bojer) Raf.; AM086721, AM086916, AM086834; Cult. at the roadside; *Marazzi & Flores BM183*, Mexico, Oaxaca, MEXU, Z.

Gleditsia sinensis Lam.; AM086719, AM086917, AM086835; Cult. in Alte Botanische Garten Z s.n.; *Marazzi BM188*, garden, Z. ***G. triacanthos*** L.; AM086720, AM086918, AM086836; Cult. in Alte Botanische Garten Z s.n.; *Marazzi BM189*, garden, Z.

Senna acclinis (F. Muell.) Randell; PS, Int; AM086721, AM086922, AM086837; Seed Bank RBGA 20020785; *Johnstone 1137*, Australia, New South Wales, SYD. ***S. aciphylla*** (Benth.) Randell; PS, Int; AM086722, AM086923, AM086838; Seed Bank RBGA 884115; *D'Aubert 432*, Australia, New South Wales, SYD. ***S. acuruensis* var. *acuruensis*** (Benth.) H.S. Irwin & Barneby; PE, Itg; AM086568, AM086586, AM086604; Wild; *Queiroz & Marazzi LQ 9198*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *acuruensis*** (Benth.) H.S. Irwin & Barneby; PE, Itg; AM086569, AM086587, —; Wild; *Queiroz & Marazzi LQ 9201*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *catingae*** (Harms) H.S. Irwin & Barneby; PE, Itg; AM086571, AM086589, AM086606; Wild; *Queiroz & Marazzi LQ 9173*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *catingae*** (Harms) H.S. Irwin & Barneby; PE, Itg;

AM086570, AM086588, AM086605; Wild; *Queiroz & Marazzi LQ 9177*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *catingae*** (Harms) H.S. Irwin & Barneby; PE, Itg; AM086572, AM086590, AM086607; Wild; *Queiroz & Marazzi LQ 9205*, Brazil, Bahia, HUEFS, Z. ***S. alata*** (L.) Roxb.; SE, Pic; —, AM086924, AM086839; Wild; *Marazzi & al. BM026*, Paraguay, Caaguazú, PY, CTES, Z. ***S. andrieuxii*** (Benth.) H.S. Irwin & Barneby; PE, Des; AM086723, AM086925, AM086840; Wild; *Marazzi & Flores BM162*, Mexico, Puebla, MEXU, Z. ***S. aphylla*** (Cav.) H.S. Irwin & Barneby; PE, Aph; AM086724, AM086926, AM086841; Wild; *Marazzi et al. BM084*, Argentina, Santiago del Estero, CTES, Z. ***S. apiculata*** (M. Martens & Galeotti) H.S. Irwin & Barneby; CH, Bra; AM086725, AM086927, AM086842; Wild; Marazzi & Flores BM170, Mexico, Puebla, MEXU, Z. ***S. argentea*** (Kunth) H.S. Irwin & Barneby; CH, Bra; AM086726, AM086928, AM086843; Wild; *Marazzi & Flores BM175*, Mexico, Oaxaca, MEXU, Z. ***S. armata*** (S. Watson) H.S. Irwin & Barneby; CH, Arm; AM086727, AM086929, AM086844; Wild; *Schönenberger JS751*, USA, California, Z[a]. ***S. artemisioides*** (DC.) Randell; PS, Sub; AM086728, AM086919, AM086845; Cult. s.n. Z; *Marazzi BM002*, garden, Z. ***S. atomaria*** (L.) H.S. Irwin & Barneby; PE, Des; AM086729, AM086930, AM086846; Wild; *Marazzi & Flores BM173*, Mexico, Oaxaca, MEXU, Z. ***S. aversiflora*** (Herbert) H.S. Irwin & Barneby; PE, Itg; AM086573, AM086591, — ; Wild; *Queiroz & Marazzi LQ 9204*, Brazil, Bahia, HUEFS, Z. ***S. barclayana*** (Sweet) Randell; CH, Bas; AM086730, AM086931, AM086847; Cult. PBIB 2003/76; *Marazzi BM136*, garden, Z. ***S. bauhinioides*** (A. Gray) H.S. Irwin & Barneby; CH, Bra; AM086731, AM086932, AM086848; Wild; *Spellenberg & Brouillet 12700*, USA, New Mexico, MT, NMC. ***S. bicapsularis*** (L.) Roxb.; CH, Col; AM086732, AM086933, AM086849; Wild; *Marazzi & Alvárez BM159*, Republic of Panama, Coclé, PMA, STRI, Z. ***S. birostris* var. *hookeriana*** (Hook.) H.S. Irwin & Barneby; CH, Sti; AM086733, AM086934, AM086850; Wild; *Marazzi et al. BM090*, Argentina, Tucumán, CTES, Z. ***S. cana* var. *calva*** H.S. Irwin & Barneby; CH, Lax; AM086574, AM086592, AM086608; Wild; *Conceiçao & Marazzi 1132*, Brazil, Bahia, HUEFS, Z. ***S. cardiosperma*** (F. Muell.) Randell; PS, Sub; AM086734, AM086935, AM086851; Seed Bank KPBG 952264; *Sweedman S2938*, Australia, Western Australia, KPBG, PERTH. ***S. cernua*** (Balb.) H.S. Irwin & Barneby; CH, Bas; AM086735, AM086936, AM086852; Wild; *Marazzi et al. BM007*, Paraguay, Caaguazú, PY, CTES, Z. ***S. chacoënsis*** (L. Bravo) H.S. Irwin & Barneby; PE, Aph; AM086736, AM086937, AM086853; Wild; *Marazzi et al. BM083*, Argentina, Santiago del Estero, CTES, Z. ***S. chloroclada*** (Harms) H.S. Irwin & Barneby; PE, Chl; AM086737, AM086938, AM086854; Wild; *Marazzi et al. BM128*, Argentina, Salta, CTES, Z. ***S. coronilloides*** (Benth.) Randell; PS, Int; AM086738, AM086939, AM086855; Seed Bank RBGA 842721; *Rodd 4219*, Australia, Queensland, SYD. ***S. corymbosa*** (Lam.) H.S. Irwin & Barneby; CH, Col; AM086739, AM086940, AM086856; Cult. in private garden; *Marazzi et al. BM103*, Argentina, Tucumán, CTES, Z. ***S. crassiramea*** (Benth.) H.S. Irwin & Barneby; PE, Aph; AM086740, AM086941, AM086857; Wild; *Marazzi et al. BM120*, Argentina, Jujuy, CTES, Z. ***S. crotalarioides*** (Kunth) H.S. Irwin & Barneby; CH, Bra; AM086741, AM086942, AM086858; Wild; *Marazzi & Flores BM163*, Mexico, Puebla, MEXU, Z. ***S. dariensis* var. *hypoglauca*** H.S. Irwin & Barneby; CH, Bac; AM086742, AM086943, AM086859; Wild; *Marazzi & Alvárez BM153*, Republic of Panama, Coclé, PMA, STRI, Z. ***S. didymobotrya*** (Fresen.) H.S. Irwin & Barneby; SE, Pic; AM086743, AM086920, AM086860; Cult. Z 19700009; *Marazzi BM002*, garden, Z. ***S. galeottiana*** (M. Martens) H.S. Irwin & Barneby; CH, Gal; AM086744, AM086944, AM086861; Wild; *Marazzi & Flores BM165*, Mexico, Puebla, MEXU, Z. ***S. gardneri*** (Benth.) H.S. Irwin & Barneby; CH, Bac; AM086575, AM086593, AM086609; Wild; *Queiroz LQ 7866*; Brazil, Bahia, HUEFS. ***S. glutinosa*[b]** (DC.) Randell; PS, Sub; AM086745, AM086945, AM086862; Seed Bank KPBG 952287; *Demarz 4803*, Australia, Western Australia, unk. ***S. hayesiana*** (Britton & Rose) H.S. Irwin & Barneby; CH, Bac; AM086746, AM086946, AM086863; Wild; *Marazzi & Alvárez BM150*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. herzogii*** (Harms) H.S. Irwin & Barneby; CH, Bac; AM086747, AM086947, AM086864;

Wild; *Morrone & Belgrano 5084*, Bolivia, Santa Cruz, LPB, SI, CTES. ***S. hilariana*** (Benth.) H.S. Irwin & Barneby; CH, Col; AM086748, AM086948, AM086865; Wild; *Marazzi et al. BM027*, Paraguay, Alto Paraná, PY, CTES, Z. ***S. hirsuta* var. *hirta*** (Benth.) H.S. Irwin & Barneby; CH, Bas; AM086749, AM086949, AM086866; Wild; *Marazzi & Flores BM168*, Mexico, Puebla, MEXU, Z. ***S. hirsuta* var. *hirta*** (Benth.) H.S. Irwin & Barneby; CH, Bas; AM086750, AM086950, AM086867; Wild; *Marazzi et al. BM115*, Argentina, Salta, CTES, Z. ***S. hirsuta* var. *leptocarpa*** (Benth.) H.S. Irwin & Barneby; CH, Bas; AM086751, AM086951, AM086868; Wild; *Marazzi et al. BM065*, Paraguay, San Pedro, PY, CTES, Z. ***S. holwayana* var. *holwayana*** (Rose) H.S. Irwin & Barneby; PE, Itg; AM086752, AM086952, AM086869; Wild; *Marazzi & Flores BM161*, Mexico, Puebla, MEXU, Z. ***S.* indet. ser. *Bacillares***; CH, Bac; AM086753, AM086953, AM086870; Wild; *Marazzi & Alvárez BM160*, Republic of Panama, Panamá, PMA, STRI, Z. ***S.* indet. ser. *Subverrucosae*[c]**; PS, Sub; AM086754, AM086954, AM086871; Seed Bank KPBG 952265; *Demarz 5523*, Australia, Western Australia, unk. ***S. italica*** Mill.; SE, Sen; AM086755, AM086955, AM086872; Wild; *Zietsmann 4345*, Republic of South Africa, Free State, NMB, Z. ***S. macranthera* var. *nervosa*** (Vogel) H.S. Irwin & Barneby; CH, Bac; AM086756, AM086956, AM086873; Cult. at the roadside; *Marazzi et al. BM082*, Paraguay, Caaguazú, PY, CTES, Z. ***S. magnifolia*** (F. Muell.) Randell; SE, Pic; AM086757, AM086957, AM086874; Seed Bank RBGA 861394; *Rodd 4526*, Australia, Western Australia, unk. ***S. martiana*** (Benth.) H.S. Irwin & Barneby; SE, Pic; AM086576, AM086594, AM086610; Wild; *Queiroz LQ 7916*, Brazil, Bahia HUEFS. ***S. mexicana*** (Jacq.) H.S. Irwin & Barneby; CH, Bas; AM086758, AM086958, AM086875; Cult. BGM 96/3360; *Marazzi BM006*, garden, Z. ***S. mollissima*** (Willd.) H.S. Irwin & Barneby; PE, Des; AM086759, AM086959, AM086876; Wild; *Marazzi & Flores BM181*, Mexico, Oaxaca, MEXU, Z. ***S. morongii*** (Britton) H.S. Irwin & Barneby; CH, Col; AM086760, AM086960, AM086877; Wild; *Marazzi et al. BM130*, Argentina, Salta, CTES, Z. ***S. mucronifera*** (Benth.) H.S. Irwin & Barneby; CH, Tri; AM086761, AM086961, AM086878; Wild; *Marazzi et al. BM019*, Paraguay, Caaguazú, PY, CTES, Z. ***S. multijuga* var. *lindleyana*** (Gardner) H.S. Irwin & Barneby; PE, Itg; AM086577, AM086595, AM086611; Wild; *Queiroz & Marazzi LP 9226*, Brazil, Bahia, HUEFS, Z. ***S. multijuga* var. *multijuga*** (Rich.) H.S. Irwin & Barneby; PE, Itg; AM086762, AM086962, AM086879; Cult. in private garden; *Marazzi & Alvárez BM151*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. nicaraguensis*** (Benth.) H.S. Irwin & Barneby; SE, Pic; AM086763, AM086963, AM086880; Wild; *Marazzi & Flores BM185*, Mexico, Chiapas, MEXU, Z. ***S. notabilis*** (F. Muell.) Randell; SE, Pic; AM086764, AM086964, AM086881; Seed Bank RBGA 872886; *Johnstone 37*, Australia, Northern Territory, SYD. ***S. obtusifolia*** (L.) H.S. Irwin & Barneby; CH, Tri; AM086765, AM086965, AM086882; Wild; *Marazzi et al. BM024*, Paraguay, Caaguazú, PY, CTES, Z. ***S. occidentalis*** (L.) Link; CH, Bas; AM086766, AM086966, AM086883; Wild; *Marazzi et al. BM060*, Paraguay, Caaguazú, PY, CTES, Z. ***S. odorata*** (Morris) Randell; PS, Int; AM086767, AM086967, — ; Cult. ANBG 68349; s.n., garden, CBG. ***S. oligoclada*** (F. Muell.) Randell; PS, Oli; AM086768, AM086968, AM086884; Seed Bank RBGA 880070; collector unk., Australia, unk. ***S. pallida*** (Vahl) H.S. Irwin & Barneby; PE, Itg; AM086769, AM086969, AM086885; Wild; *Marazzi & Flores BM178*, Mexico, Oaxaca, MEXU, Z. ***S. paradictyon*** (Vogel) H.S. Irwin & Barneby; PA; AM086770, AM086970, AM086886; Wild; *Marazzi et al. BM028*, Paraguay, Alto Paraná, PY, CTES, Z. ***S. pendula*** (Willd.) H.S. Irwin & Barneby; CH, Col; AM086771, AM086971, AM086887; Wild; *Marazzi et al. BM117*, Argentina, Salta, CTES, Z. ***S. phlebadenia*** H.S. Irwin & Barneby; CH, Lax; AM086578, AM086596, AM086612; Wild; *Stapf 209*, Brazil, Bahia, HUEFS. ***S. pilifera*** (Vogel) H.S. Irwin & Barneby; CH, Tri; AM086772, AM086972, AM086888; Wild; *Marazzi et al. BM011*, Paraguay, Caaguazú, PY, CTES, Z. ***S. pinheiroi*** H.S. Irwin & Barneby; CH, Bac; AM086579, AM086597, AM086613; Wild; *Queiroz 9210*, Brazil, Bahia, HUEFS. ***S. pleurocarpa*** (F. Muell.) Randell; SE, Pic; AM086773, AM086973, AM086889; Seed Bank KPBG 930575; *Demarz 12081*, Australia, Western Australia, unk. ***S. polyantha*** (Collad.)

H.S. Irwin & Barneby; CH, Gal; AM086774, AM086974, AM086890; Wild; *Marazzi & Flores BM172*, Mexico, Oaxaca, MEXU, Z. ***S. purpusii*** (Brandegee) H.S. Irwin & Barneby; CH, Pac; AM086775, AM086921, AM086891; Cult. BGB 3585/96-P; *Marazzi BM004*, garden, Z. ***S. quinquangulata*** (Rich.) H.S. Irwin & Barneby; CH, Bac; AM086580, AM086598, AM086614; Wild; *Queiroz & Marazzi LQ 9220*, Brazil, Bahia, HUEFS, Z. ***S. reticulata*** (Willd.) H.S. Irwin & Barneby; SE, Pic; AM086776, AM086975, AM086892; Wild; *Marazzi & Alvárez BM154*, Republic of Panama, Coclé, PMA, STRI, Z. ***S. rigida*** (Hieron.) H.S. Irwin & Barneby; PE, Aph; AM086777, AM086976, AM086893; Wild; *Marazzi et al. BM108*, Argentina, Salta, CTES, Z. ***S. cf. rigida***; PE, Aph; AM086778, AM086977, AM086894; Wild; *Marazzi et al. BM104*, Argentina, Salta, CTES, Z. **S. rizzinii** H.S. Irwin & Barneby; CH, Bac; AM086581, AM086599, AM086615; Wild; Conceição & Marazzi 1126, Brazil, Bahia; HUEFS, Z. ***S. robiniifolia*** (Benth.) H.S. Irwin & Barneby; PE, Itg; AM086779, AM086978, AM086895; Cult. BGM 98/3500w; *Marazzi BM005*, garden, Z. ***S. rugosa*** (G. Don) H.S. Irwin & Barneby; CH, Bac; AM086582, AM086600, AM086616; Wild; *Giulietti 2337*, Brazil, Bahia, HUEFS. ***S. septemtrionalis*** (Viviani) H.S. Irwin & Barneby; CH, Col; AM086780, AM086979, AM086896; Cult. BGM s.n.; *Marazzi BM140*, garden, Z. ***S. siamea*** (Lam.) H.S. Irwin & Barneby; CH, Flo; AM086781, AM086980, AM086897; Cult., Causeway, Panamá City; *Marazzi & Alvárez BM157*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. silvestris* var. *guaranitica*** (Chodat & Hassl.) H.S. Irwin & Barneby; CH, Sap; AM086782, AM086981, AM086898; Wild; *Marazzi et al. BM068*, Paraguay, San Pedro, PY, CTES, Z. ***S. skinneri*** (Benth.) H.S. Irwin & Barneby; CH, Ski; AM086783, AM086982, AM086899; Wild; *Marazzi & Flores BM176*, Mexico, Oaxaca, MEXU, Z. ***S. spectabilis*** (DC.) H.S. Irwin & Barneby; PE, Exc; AM086784, AM086983, AM086900; Wild; *Marazzi et al. BM029*, Paraguay, Alto Paraná, PY, CTES, Z. ***S. splendida* var. *gloriosa*** H.S. Irwin & Barneby; CH, Bac; AM086583, AM086601, AM086617; Wild; *Araújo 1566*, Brazil, Ceará, HUEFS. ***S. subulata*** (Griseb.) H.S. Irwin & Barneby; CH, Col; AM086785, AM086984, AM086901; Wild; *Potzner & Belgrano 427*, Argentina, Jujuy, SI. ***S. tonduzii*** (Standl.) H.S. Irwin & Barneby; PE, Itg; AM086786, AM086985, AM086902; Wild; *Marazzi & Flores BM187*, Mexico; Chiapas, MEXU, Z. ***S. uniflora*** (Mill.) H.S. Irwin & Barneby; CH, Con; AM086787, AM086986, AM086903; Wild; *Marazzi & Flores BM186*, Mexico, Chiapas, MEXU, Z. ***S. unijuga*** (Rose) H.S. Irwin & Barneby; PE, Des; AM086788, AM086987, AM086904; Wild; *Marazzi & Flores BM167*, Mexico, Puebla, MEXU, Z. ***S.* cf. *velutina*** (Vogel) H.S. Irwin & Barneby; CH, Lax; AM086789, AM086988, AM086905; Wild; *Morrone & Belgrano 4988*, Bolivia, Santa Cruz, LPB, SI, CTES. ***S. venusta*** (F. Muell.) Randell; SE, Pic; — , AM086989, AM086906; Seed Bank RBGA 881196; *McCarthy 224*, Australia Western Australia, unk. ***S. villosa*** (Mill.) H.S. Irwin & Barneby; AS; AM086790, AM086990, AM086907; Wild; *Marazzi & Flores BM174*, Mexico, Oaxaca, MEXU, Z. ***S. williamsii*** (Britton & Rose) H.S. Irwin & Barneby; PE, Itg; AM086791, AM086991, AM086908; Wild; *Marazzi & Alvárez BM158*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. wislizeni*** (A. Gray) H.S. Irwin & Barneby; PE, Des; AM086792, AM086992, AM086909; Wild; *Marazzi & Flores BM169*, Mexico, Puebla, MEXU, Z.

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[a] Voucher: fixed material in 70% ethanol.

[b] *Senna glutinosa*: plant material received with the name *Senna* form *glutinosa*.

[c] *Senna* indet. ser. *Subverrucosae*: plant material received with the invalid species name of *S. nemophila* most likely corresponding to *Senna* form '*oligophylla*' (R. Johnstone, Royal Botanic Garden Mount Annan, N.S.W., Australia, personal communication).

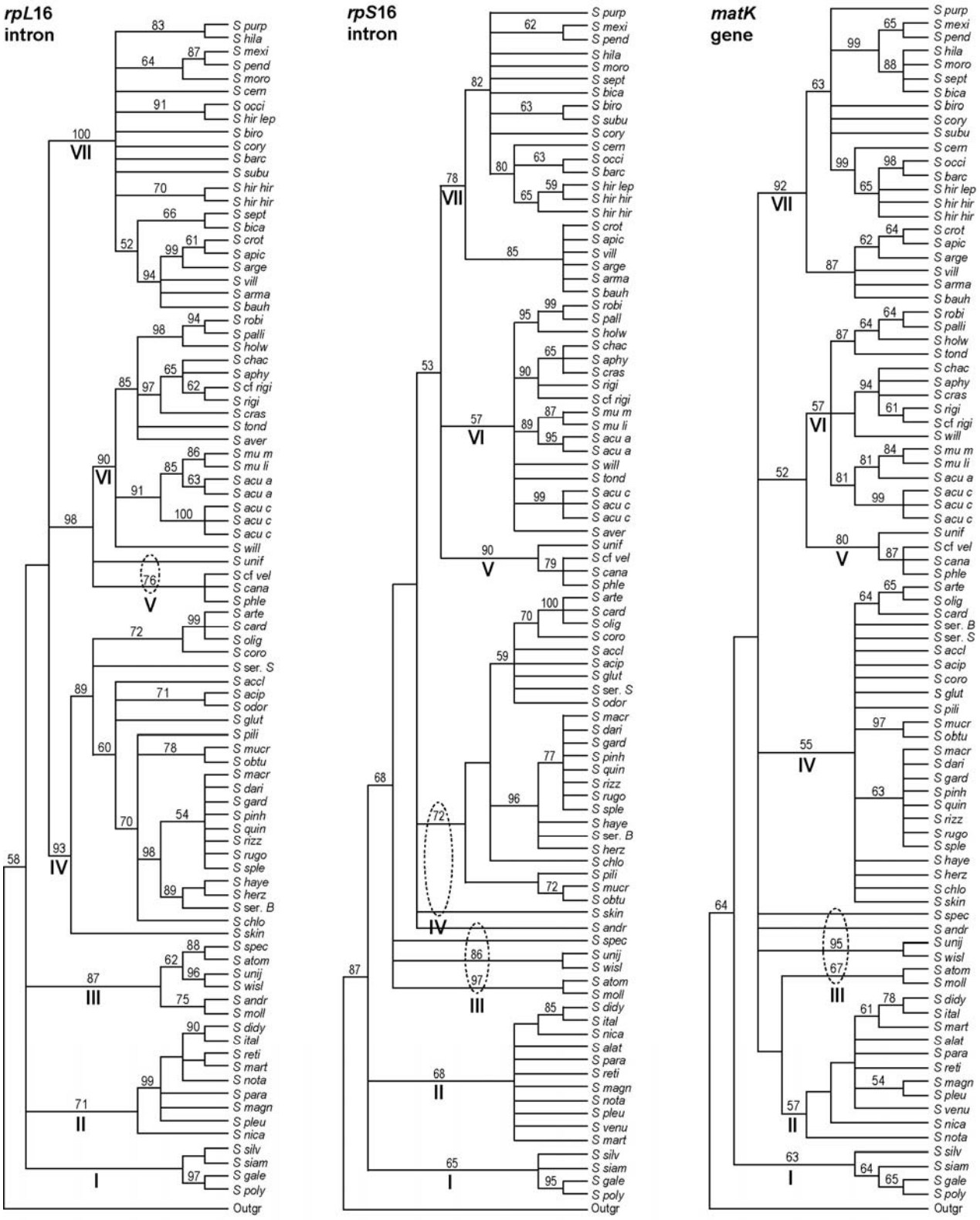
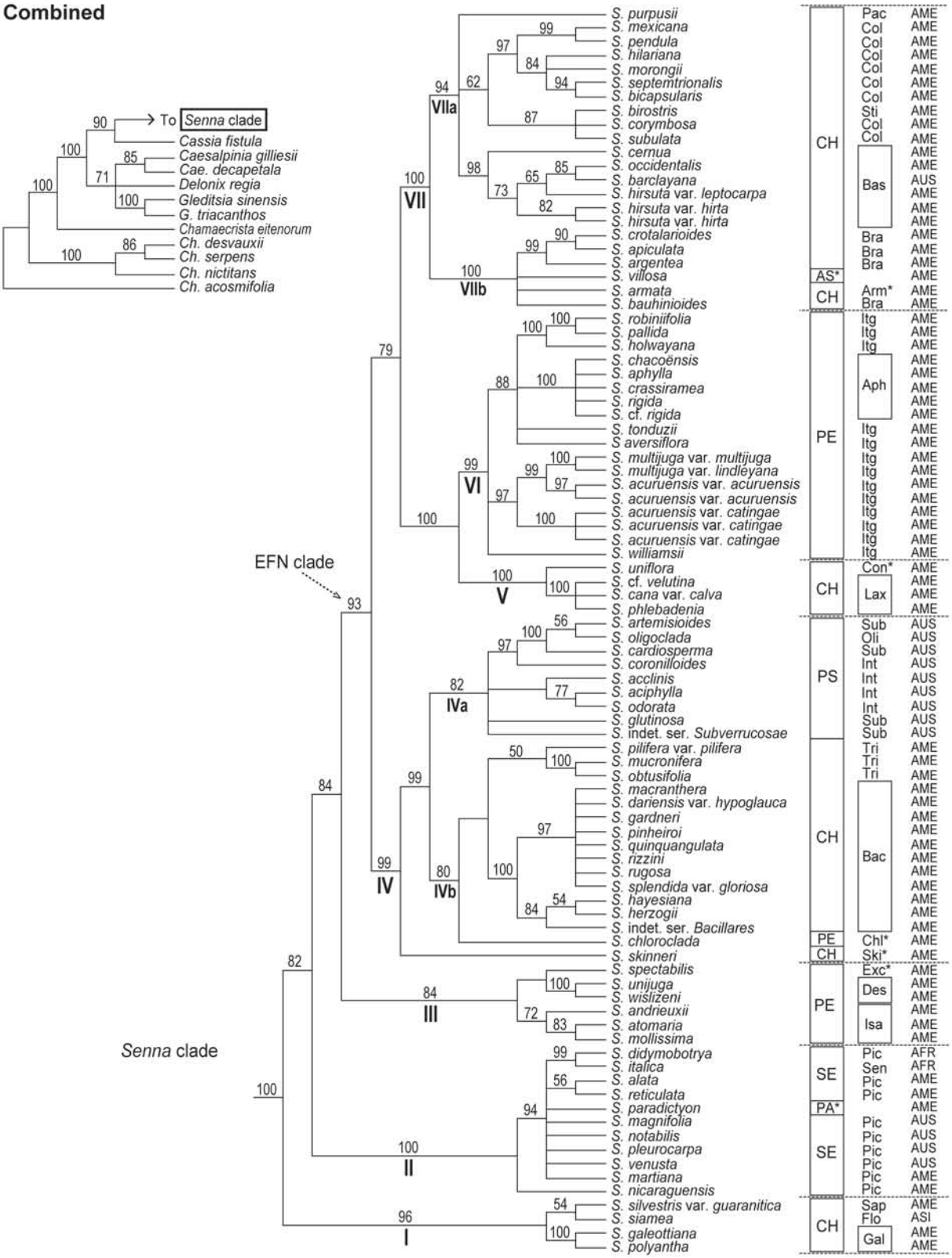


Fig. 1

Fig. 1 Strict consensus trees resulting from parsimony analyses of each individual matrix (see Table 2). The outgroup species (Outgr) are listed in fig. 2. Bold Roman numerals indicate major clades; dotted ellipses show the taxa that form a clade in the combined tree (fig. 2).

Fig. 2 Strict consensus tree resulting from parsimony analyses of the combined matrix (see Table 2). Bootstrap support [BS] values >50% are reported above the branches. Bold Roman numerals below the branches indicate major clades and subclades. *Senna* species, sections, series, and geographic distribution are listed to the right of the tree (see Appendix 1 for full section and series names; AFR, Africa; AME, America; ASI, Asia; AUS, Australia). Monophyletic series are framed; monotypic sections and series are marked with an asterisk. Dashed horizontal lines show delimitations of the major clades. Outgroups are listed in the partial tree on the left.


Combined
To Senna clade
Cassia fistula
Caesalpinia gilliesii
Cae. decapetala
Delonix regia
Gleditsia sinensis
G. triacanthos
Chamaecrista eitenorum
Ch. desvauxii
Ch. serpens
Ch. nictitans
Ch. acosmifolia
EFN clade
Senna clade
S. purpusii
S. mexicana
S. pendula
S. hilariana
S. morongii
S. septemtrionalis
S. bicapsularis
S. birostris
S. corymbosa
S. subulata
S. cernua
S. occidentalis
S. barclayana
S. hirsuta var. leptocarpa
S. hirsuta var. hirta
S. hirsuta var. hirta
S. crotalarioides
S. apiculata
S. argentea
S. villosa
S. armata
S. bauhinioides
S. robiniifolia
S. pallida
S. holwayana
S. chacoënsis
S. aphylla
S. crassiramea
S. rigida
S. cf. rigida
S. tonduzii
S aversiflora
S. multijuga var. multijuga
S. multijuga var. lindleyana
S. acuruensis var. acuruensis
S. acuruensis var. acuruensis
S. acuruensis var. catingae
S. acuruensis var. catingae
S. acuruensis var. catingae
S. williamsii
S. uniflora
S. cf. velutina
S. cana var. calva
S. phlebadenia
S. artemisioides
S. oligoclada
S. cardiosperma
S. coronilloides
S. acclinis
S. aciphylla
S. odorata
S. glutinosa
S. indet. ser. Subverrucosae
S. pilifera var. pilifera
S. mucronifera
S. obtusifolia
S. macranthera
S. dariensis var. hypoglauca
S. gardneri
S. pinheiroi
S. quinquangulata
S. rizzini
S. rugosa
S. splendida var. gloriosa
S. hayesiana
S. herzogii
S. indet. ser. Bacillares
S. chloroclada
S. skinneri
S. spectabilis
S. unijuga
S. wislizeni
S. andrieuxii
S. atomaria
S. mollissima
S. didymobotrya
S. italica
S. alata
S. reticulata
S. paradictyon
S. magnifolia
S. notabilis
S. pleurocarpa
S. venusta
S. martiana
S. nicaraguensis
S. silvestris var. guaranitica
S. siamea
S. galeottiana
S. polyantha
VIIa
VII
VIIb
VI
V
IVa
IVb
IV
III
II
I
CH
Pac AME
Col AME
Col AME
Col AME
Col AME
Col AME
Col AME
Col AME
Sti AME
Col AME
Col AME
Bas
AUS
AME
Bra AME
Bra AME
Bra AME
AS* AME
CH Arm* AME
Bra AME
PE
Itg AME
Itg AME
Itg AME
Aph
AME
AME
AME
AME
AME
Itg AME
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CH
Con* AME
Lax
AME
AME
AME
PS
Sub AUS
Oli AUS
Sub AUS
Int AUS
Int AUS
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Int AUS
Sub AUS
Sub AUS
CH
Tri AME
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Tri AME
Bac
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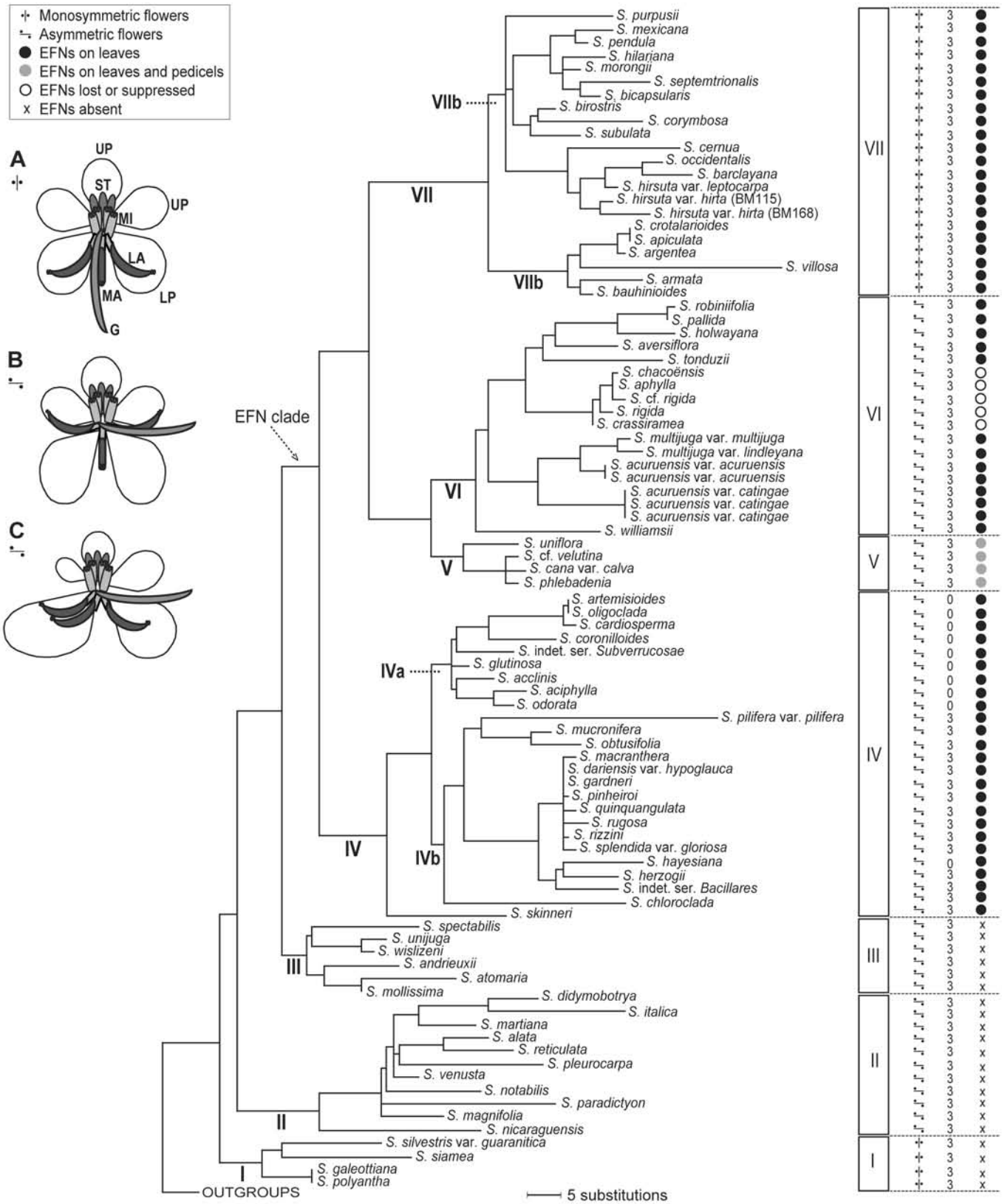
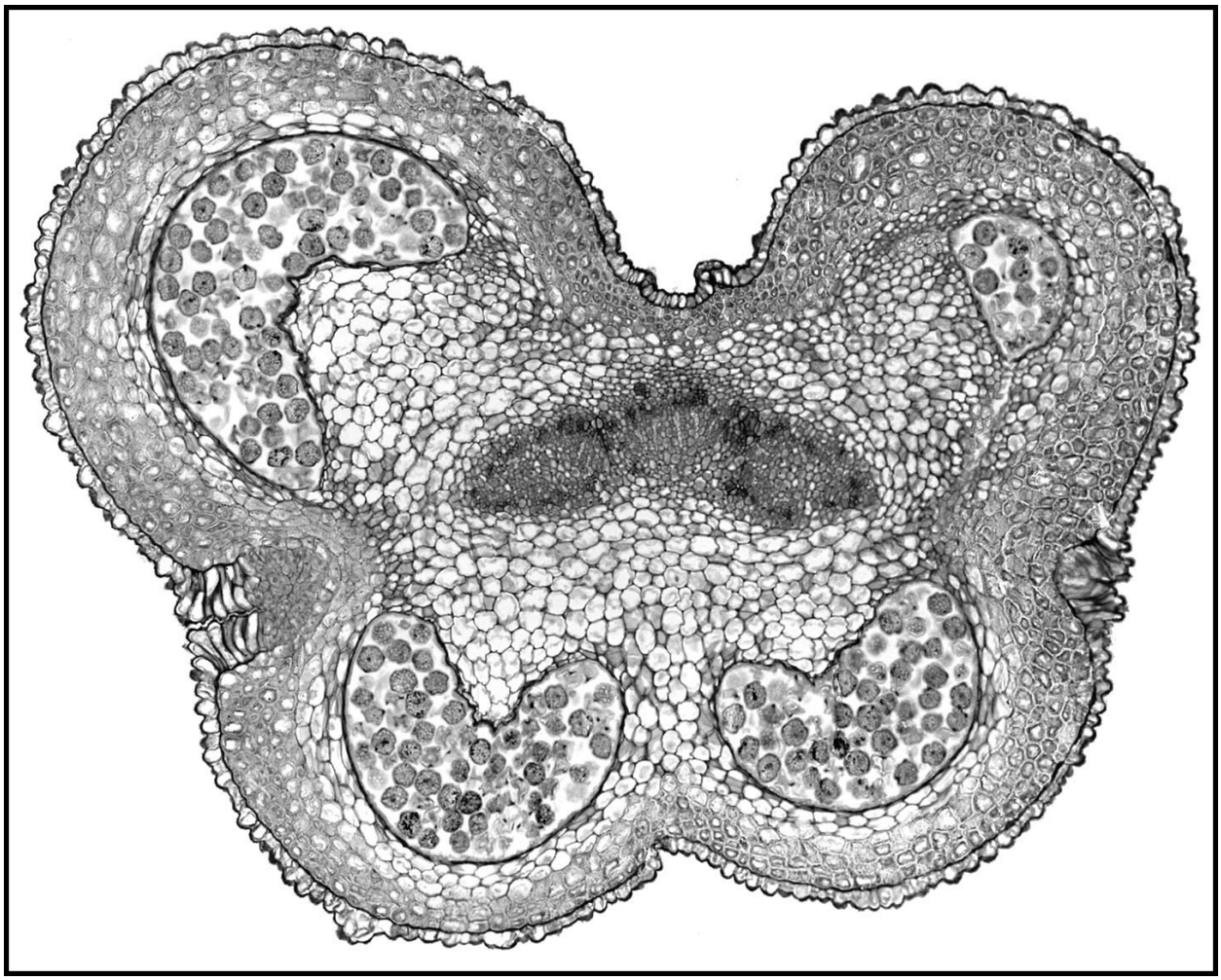
PE Chl* AME
CH Ski* AME
PE
Exc* AME
Des
AME
AME
Isa
AME
AME
SE
Pic AFR
Sen AFR
Pic AME
Pic AME
PA* AME
SE
Pic AUS
Pic AUS
Pic AUS
Pic AUS
Pic AME
Pic AME
CH
Sap AME
Flo ASI
Gal
AME
AME

Fig. 3 One of the MP trees resulting from the analyses of the combined matrix (1578 steps; CI = 0.674; RI = 0.840; all characters included). Bold Roman numerals below the branches indicate major clades and subclades. A branch-length scale is reported below the tree. Outgroups are listed in fig. 2. The symbols to the right of the boxes representing the major clades indicate, respectively: floral symmetry, number of adaxial staminodes, extrafloral nectaries (EFNs). Dashed horizontal lines show delimitations of the major clades. A–C. schematic diagrams of floral symmetry patterns in *Senna* species: (A) Monosymmetric flower (*S. silvestris*, sect. *Chamaefistula*, clade I); AL, abaxial lateral stamen; AM, abaxial median stamen; G, gynoecium; LP, lower petal; MI, set of four middle stamens; ST, set of three adaxial staminodes; UP, upper petal; sepals not shown. (B) Asymmetric flower in which only the gynoecium is involved in the floral asymmetry (*S. nicaraguensis*, sect. *Senna*, clade II). (C) Asymmetric flower in which also petals and stamens are involved in the floral asymmetry (*S. pallida*, sect. *Peiranisia*, clade VI).


Monosymmetric flowers
Asymmetric flowers
EFNs on leaves
EFNs on leaves and pedicels
EFNs lost or suppressed
x EFNs absent
A
UP
ST
UP
MI
LA
MA
LP
G
B
C
EFN clade
VIIb
VII
VIIb
VI
V
IVa
IV
IVb
III
II
I
OUTGROUPS
S. purpusii
S. mexicana
S. pendula
S. hilariana
S. morongii
S. septemtrionalis
S. bicapsularis
S. birostris
S. corymbosa
S. subulata
S. cernua
S. occidentalis
S. barclayana
S. hirsuta var. leptocarpa
S. hirsuta var. hirta (BM115)
S. hirsuta var. hirta (BM168)
S. crotalarioides
S. apiculata
S. argentea
S. villosa
S. armata
S. bauhinioides
S. robiniifolia
S. pallida
S. holwayana
S. aversiflora
S. tonduzii
S. chacoënsis
S. aphylla
S. cf. rigida
S. rigida
S. crassiramea
S. multijuga var. multijuga
S. multijuga var. lindleyana
S. acuruensis var. acuruensis
S. acuruensis var. acuruensis
S. acuruensis var. catingae
S. acuruensis var. catingae
S. acuruensis var. catingae
S. williamsii
S. uniflora
S. cf. velutina
S. cana var. calva
S. phlebadenia
S. artemisioides
S. oligoclada
S. cardiosperma
S. coronilloides
S. indet. ser. Subverrucosae
S. glutinosa
S. acclinis
S. aciphylla
S. odorata
S. pilifera var. pilifera
S. mucronifera
S. obtusifolia
S. macranthera
S. dariensis var. hypoglauca
S. gardneri
S. pinheiroi
S. quinquangulata
S. rugosa
S. rizzini
S. splendida var. gloriosa
S. hayesiana
S. herzogii
S. indet. ser. Bacillares
S. chloroclada
S. skinneri
S. spectabilis
S. unijuga
S. wislizeni
S. andrieuxii
S. atomaria
S. mollissima
S. didymobotrya
S. italica
S. martiana
S. alata
S. reticulata
S. pleurocarpa
S. venusta
S. notabilis
S. paradictyon
S. magnifolia
S. nicaraguensis
S. silvestris var. guaranitica
S. siamea
S. galeottiana
S. polyantha
5 substitutions
VII
VI
V
IV
III
II
I

PART 2

FLORAL DIVERSITY: ANTHERS AND STIGMAS



Transverse section of an abaxial anther of *Senna tonduzii*.

DIVERSITY IN ANTHERS AND STIGMAS IN THE BUZZ-POLLINATED GENUS *SENNA* (LEGUMINOSAE, CASSIINAE)

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ABSTRACT

The large genus *Senna* (Cassiinae, Leguminosae) is an outstanding example of floral structural specialization associated with buzz pollination. This specialization is expressed especially in the androecium (with a high diversity of anther elaborations) and gynoecium (with diversity in stigma shape). The floral structure of 69 species from all major clades of *Senna* was studied, focusing on heteranthery, anther dehiscence, pore position, extension of the lateral furrow of the thecae, cell wall thickening in the anther tip, and stigma diversity. Filament union is reported for the first time in the genus; it involves the seven adaxial androecial organs, a pattern unique in legumes. Our investigations identified novel morphological characteristics that are congruent with the clades supported by the molecular phylogeny. Anthers of abaxial stamens with the least differentiated dehiscence pattern, i.e., two separate pores and separate thecae, are found in most major clades (I, III-V, VII). Anthers with apically confluent thecae, forming a shared chamber, and/or with a single pore by confluence of two pores, represent specialized patterns (clades II, IV, VI, VII). Diverse anther tips may reflect different strategies of pollen dispersal; anther pore position may influence pollen flow directions. Anther tip elongation in the abaxial stamens and constriction between the thecae and the anther tip may influence the speed and/or amount of the released pollen.

KEY WORDS

Poricidal anther dehiscence, buzz pollination, functional morphology, heteranthery, pollen dispersal, stamen union

INTRODUCTION

Senna (Cassiinae, Leguminosae) is a large, diverse and widespread genus of about 350 species with showy yellow nectarless flowers, which are buzz-pollinated by pollen-collecting bees. They display an array of floral traits typically related to the specialized pollination biology, including heteranthery (i.e., different kinds of stamens in a flower), poricidal anthers (i.e., dehiscence restricted to apical pores), point-tipped stigmas, enantiostyly (i.e., deflection of the single carpel to the left or right) and asymmetric corolla and androecium (Buchmann 1974; Delgado Salinas and Souza Sánchez 1977; Dulberger 1981; Irwin and Barneby 1981; Gottsberger and Silberbauer-Gottsberger 1988; Owens and Lewis 1989; Dulberger et al. 1994). In addition to species with enantiostylous, asymmetric flowers, there are also species with monosymmetric (zygomorphic) flowers.

Although the specialized floral traits of *Senna* have intrigued researchers for a long time (Müller 1883; Burck 1886), their role in pollination biology has been investigated only in a few species (Buchmann 1974; Delgado Salinas and Souza Sanchéz 1977; Fontanelle 1979; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003; Laporta 2003). This is also true for their floral morphology, in which specializations of the stigma (Owens and Lewis 1989; Dulberger et al. 1994), structure of the poricidal stamens (Venkatesh 1957; Lasseigne 1979; Endress 1994; Tucker 1996a), and floral development (Tucker 1996b) were studied. Most of these studies compare *Senna* with *Cassia* L. and *Chamaecrista* Moench (the three genera belonging to subtribe Cassiinae - formerly known as *Cassia* s.l.; Irwin and Barneby 1981, 1982), all usually characterized by yellow and nectarless, buzzed flowers (Delgado Salinas and Souza Sanchéz 1977; Gottsberger and Silberbauer-Gottsberger 1988). Such flowers are otherwise unusual in legumes. The evolution of floral diversity in *Senna* is highly intriguing and still enigmatic. Molecular phylogenetic work by Marazzi et al. (2006) does not support the traditional interpretations of morphological evolution and classification systems of *Senna* (Irwin and Barneby 1982; Randell 1988, 1989, 1990; Singh 2001; see Marazzi et al. 2006, Table 1). The present work is part of a larger project aimed at understanding floral evolution within *Senna*, and focuses on stamen and gynoecium diversity in the genus, whereas floral symmetry will be treated elsewhere.

The androecium and, in particular, the poricidal anther tips have been recognized as the most diverse and interesting floral parts of *Senna* (Venkatesh 1957; Lasseigne 1979; Fontanelle 1979; Irwin and Barneby 1981; Tucker 1996a). The typically five-merous flowers of *Senna* have two androecial whorls with up to four different kinds of stamens. The three adaxial stamens (1) are usually staminodial, and produce pollen only in the species of *Senna* section *Psilorhegma* (Irwin and Barneby 1981). The remaining fertile stamens form (2) a set of four middle ‘feeding’ stamens (with pollen collected for larval provision) and (3) a set of three usually longer abaxial ‘pollinating’ stamens (with pollen for pollination), in which (4) the median stamen may be smaller than the lateral ones, highly reduced and sterile or even absent in several species. Anthers are slightly dorsifixed, with apical dehiscence through two separate pores or two pores that are confluent into one pore. A more or less deep lateral furrow (non-functional stomium) usually runs from the lower end of each dehiscence pore down along the entire length of each theca. In many species, the anthers are variably beaked, i.e. have a more or less elongated sterile anther tip (Venkatesh 1957; Lasseigne 1979). Some *Senna* species have unusual, long tubular beaks, not found in other buzzed caesalpinioids, and indeed rare in buzz-pollinated angiosperms.

As characteristic in legumes, flowers of *Senna* have a single carpel, usually more or less curved upwards and with a short stipe. Although the gynoecium is less diverse than the androecium, there is some variability in the deflection of the carpel (not discussed here) and, in particular, in the unusual stigma structure with enclosed stigmatic surface. Dulberger (1981) first described the extremely small orifice at the end of the style. Two types of stigma have been recognized in *Senna* (and other Cassiinae): (i) a chambered type, with the receptive surface

enclosed in a cavity, the entrance of which is more or less closed; (ii) and a crater-like type, with the receptive surface not enclosed (Owens and Lewis 1989; Dulberger et al. 1994; Endress 1994). Hairs may variously fringe the entrance of the cavity, and a drop of secretion may occur at the outlet. In contrast to other angiosperms with an orifice at the end of a stylar canal, in *Senna* the cells surrounding the orifice are not part of the tissue that lines the cavity and produces the secretory material (Owens 1985; Dulberger et al. 1994). Moreover, the cuticle of these cells apparently does not allow pollen germination, which occurs in the secretion droplet within the cavity (Owens 1985; Dulberger et al. 1994).

Several features of the androecium, but almost no features of the gynoecium were used for taxonomic purposes in *Senna* (e.g., Bentham 1871, as *Cassia*; Irwin and Barneby 1982). For example, the fertility of all stamens distinguishes sect. *Psilorhegma* from all other sections in *Senna*. In the traditional interpretations of evolutionary trends in the floral morphology of the genus (Irwin and Barneby 1982), fertility of all 10 stamens and anthers with two pores represented the ancestral condition of the androecium. In contrast, Marazzi et al. (2006) suggested that flowers of *Senna* ancestrally had seven (or fewer) fertile stamens and three adaxial staminodes, the condition of the androecium traditionally considered as derived. Thus fertility of all stamens represents a synapomorphy of *Psilorhegma* that supports its monophyly (Marazzi et al. 2006). It remains unclear whether two anther pores represent the ancestral state in *Senna* and a single pore by confluence of two the derived state. The orientation of the abaxial stamens used to distinguish sect. *Senna* from sect. *Chamaefistula* by Irwin and Barneby (1982) is characteristic for clade II of *Senna* in the study by Marazzi et al. (2006), which includes the species of sect. *Senna* and *S. paradictyon* of the (no longer monotypic) sect. *Paradictyon*. In these flowers the two lateral long abaxial anthers are curved laterally, so that their anthers face each other, resembling the arms of tongs. Section *Chamaefistula* is polyphyletic (Marazzi et al. 2006). Based on stigma morphology, Owens and Lewis (1989) divided their examined *Senna* species into five groups, which were, however, not congruent with any of Irwin and Barneby's (1982) sections or series. Heteranthery and poricidal dehiscence have not yet been explored in the genus in a systematic context.

Floral diversity in *Senna* is associated with the specialized pollination mode, which has been described in more or less detail in a number of studies. Except for the occurrence of apomixis in the *S. artemisioides* species complex (as *Cassia nemophila* group; Randell 1970) and self-compatibility in *S. silvestris* (Carvalho and Oliveira 2003), *Senna* species are generally self-incompatible, thus requiring a pollinator to set seeds (e.g. Dulberger 1981; Fontanelle 1979; Laporta 2003). The pollinators, bees belonging mostly to *Xylocopa*, but also *Bombus*, *Centris*, *Epicharis*, and *Melipona*, extract the pollen by vibrating the flowers (i.e., buzz pollination; Buchmann 1974; Salinas Delgado and Souza Sanchéz 1977; Dulberger 1981; Fontanelle 1979; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003; Laporta 2003; van Kleunen and Johnson 2005). The thecae act as a resonating chamber, where pollen grains bounce when vibrated, gaining kinetic energy, until they are forced through the apical pore (Buchmann and Hurley 1978).

It is not clear whether the feeding anthers of *Senna* provide only feeding pollen for the bee and the pollinating anthers only pollinating pollen for plant reproduction. For example, in *S. silvestris*, the feeding pollen, significantly smaller than the pollinating pollen, is not viable for plant reproduction (Carvalho and Oliveira 2003). In *S. corymbosa*, pollen tubes of pollinating pollen grow faster than those of feeding pollen (Laporta 2003), and, in *S. didymobotrya*, all anthers are fertile and provide viable feeding pollen, suggesting no separation of anther function (Dulberger 1981).

Electrostatic forces seem to play an important role in buzz pollination (e.g. Corbet et al. 1982, 1988). The minute point-tipped stigma of *Senna* species touches or approaches the sites of the bee's body from which pollen was not removed by grooming. The ratio of the area of pollen presentation on the bee's body to the area of pollen reception on the stigma approximates 10'000 in two studied *Senna* species (Dulberger 1981), suggesting an imprecise mechanism of pollen

delivery, aided by electrostatic forces that promote pollen capture and its adhesion to the minute stigmatic tip (Dulberger et al. 1994).

The major aim of the present study is to provide new structural data on the androecium and gynoecium of *Senna*, focusing on anthers and stigmas, to understand floral diversity and evolution within the genus. We will address the following specific questions: (1) What patterns of heteranthery can be recognized in the androecium of *Senna* species? (2) What patterns can be identified in the diversity of stamens and anther dehiscence? (3) Do other types of stigma exist, in addition to chambered vs. crater-like stigmas? (4) Do the investigated features provide any synapomorphies congruent with the new infrageneric relationships supported by the molecular phylogeny of *Senna* by Marazzi et al. (2006)? (5) Do our results support traditional interpretations on the evolution of the androecium by Irwin and Barneby (1982)? (6) What are the implications of our results on anther and stigma diversity to improve our understandingof pollination and floral evolution in *Senna*?

MATERIAL AND METHODS

Our taxonomic sampling included 69 *Senna* species (one or more individuals per species), almost half of which were selected for detailed structural studies with Scanning Electron Microscopy (SEM) and serial microtome sectioning, while the other species were investigated with stereomicroscopy. Sixty-six *Senna* species represent the clades of the molecular phylogeny of the genus by Marazzi et al. (2006; based on 83 species), and the diversity of morphological patterns observed. The remaining three species, *S. chrysocarpa*, *S. hebecarpa* and *S. surattensis*, were not considered in Marazzi et al. (2006).

To evaluate the consistency of the observed morphological patterns within a species, we studied additional accessions that were available for a number of species (*S. alata*, *Vanni & Marazzi* 4516; *S. chloroclada*, *Schinini & Marazzi 35285*; *S. holwayana*, *Marazzi & Flores BM166*; *S. macranthera cf.* var. *nervosa*, *Vanni & Marazzi 4543*; *S. morongii*, *Schinini & Marazzi 35279*; *S. obtusifolia*, *Vanni & Marazzi 4514*; *S. pilifera* var. *pilifera*, *Vanni & Marazzi 4513*; *S. spectabilis* var. *spectabilis*, *Vanni & Marazzi 4530*; these accessions were not used by Marazzi et al. 2006). Data for the androecium of *S. reticulata* and *S. martiana* are from Lasseigne (1979) and from color photographs made in the field by the first author, respectively. A list of the specimens studied and voucher information is given in Table 1.

Flowers at anthesis and buds at different stages were fixed and stored in 70% ethanol. They were investigated with light microscopy (LM), including serial microtome sectioning, and SEM. Standard specimen preparation procedures were used for osmium tetroxide impregnated samples for SEM studies. Specimens for serial microtome sectioning were embedded in Kulzer's Technovit 2-hydroethyl methacrylate (Igersheim 1993) and sectioned with a Microm HM 335 rotary microtome (Microm International GmbH, Walldorf, Deutschland) and conventional microtome knife (grade D); transverse section series were cut at 7 μm, stained with ruthenium red and toluidine blue (Weber and Igersheim 1994), and mounted in Histomount on glass slides. Fixed floral material and slides are deposited at the Institute of Systematic Botany of the University of Zurich (Z), Switzerland.

RESULTS

Anther dehiscence pattern of abaxial stamens

The two lateral abaxial stamens belong to the inner androecial whorl, and the median abaxial stamen to the outer one. In this study we primarily focus on the abaxial stamens, because of the higher diversity in these organs compared to the middle stamens observed in our preliminary work and by previous researchers (Venkatesh 1957; Lasseigne 1979). In particular, we studied the lateral abaxial stamens, which are fertile in almost all species (except *S. hayesiana*, see below).

The median abaxial stamen is either similar to the lateral stamens in most *Senna* species (clades III-VI, and VIIb), or in other species it is smaller (species of clades I, VIIa), or smaller and slightly different in shape (species of clades II), or highly reduced and sterile (species of clades VIIa), or absent (*S. hayesiana*, clade IV, other species of clade VIIa).

CLADE I – Anthers of the lateral abaxial stamens have two apical dehiscence slits, or (sometimes in *S. siamea*) one V-shaped slit by confluence of two (Venkatesh 1957), and point inwards (figs. 1A, B). The lateral furrow (non-functional stomium) is continuous, i.e. runs from the lower end of each dehiscence slit down along the entire length of the theca (*S. polyantha*; fig. 1A; fig. 2A, sections v-vii), or is not continuous, i.e. it is only present along the sides of the thecae between the pollen sacs, without being connected to the slits (*S. silvestris* var. *guaranitica*), or is absent (*S. siamea*, fig. 1B). The thecae are not confluent, and pollen of each theca is released by the dehiscence slit of that theca (fig. 2A; see also *S. silvestris* in Fontanelle 1979), or through the V-shaped slit by confluence of two (Venkatesh 1957). One hypodermal cell layer adjacent to the stomial region is specialized to serve the mechanical role in dehiscence: the cells are relatively narrow, radially elongated and strongly thick-walled (fig. 2A, sections ii-vi; see also Venkatesh 1957). Other hypodermal cells in the anther are also strongly thick-walled (fig. 2A), so that a continuous hypodermal cell layer of thickened cell walls is present throughout the anther. The vascular bundle extends almost up to the upper end of the slits (fig. 2A, sections ii-vii).

CLADE II – Anthers of the lateral abaxial stamens have two dehiscence pores (*S. italica*, fig. 1C, and *S. paradictyon*, fig. 1D), or only one pore, while the other expected pore is always absent on the side away from the median plane (*S. nicaraguensis*, fig. 1E). The anther tip is bent in such a way that the pores point inwards (*S. paradictyon*, fig. 1D), or more or less upwards (*S. alata*, *S. didymobotrya*, *S. italica*, fig. 1C), or outwards (*S. nicaraguensis*, fig. E). The lateral furrow is continuous (figs. 1C, 1D, 2A), or not continuous (*S. paradictyon*, fig. 1E, fig. 2B, compare sections vi-vii). The thecae are apically confluent, forming a shared chamber by decay of the tissue, and pollen traverses this chamber and is released through two separate pores (fig. 2B) or the single pore in *S. nicaraguensis*. Although in *S. nicaraguensis* one of the two pores is not formed, its canal is still present (fig. 2C, sections ii-iv). Thick-walled specialized hypodermal cells, as described in clade I, surround most of the sterile anther tip in *S. nicaraguensis*, and, together with thick-walled hypodermal and subhypodermal cells, form an evenly thick layer throughout the anther wall (fig. 2C, sections iv-vii). *S. paradictyon* lacks the specialized elongated hypodermal cells, and the evenly thick layer throughout the anther wall is less conspicuous than in *S. nicaraguensis* (fig. 2B). The vascular bundle does not extend into the sterile anther tip (figs. 2B,C).

CLADE III – Anthers of the lateral abaxial stamens have two dehiscence slits in all studied species (see figs. 1F, G). The slits point inwards (*S. unijuga*, fig. 1F), or more or less outwards (*S. andrieuxii*, *S. atomaria*, *S. mollissima*, fig. 1H, *S. spectabilis*, *S. wislizeni*, fig. 1G). The lateral furrow is continuous (figs. 1F-G). The thecae are not confluent, and pollen of each theca is released by the dehiscence slit of that theca (fig. 2D). One layer of hypodermal cells adjacent to the stomium is specialized as described in clade I, whereas the walls of other hypodermal cells are strongly thickened throughout the anther wall (*S. mollissima*, *S. wislizeni*, fig. 2D, sections iii-vii). The vascular bundle extends into the anther tip, and, in *S. wislizeni*, it branches close to the upper end of the dehiscence slits (fig. 2D, sections iii-v).

CLADE IV – This clade is composed of *S. skinneri* and a clade comprising subclades IVa and IVb (Marazzi et al. 2006). Species of subclade IVa have all the stamens fertile, whereas *S. skinneri* and species of subclade IVb have seven (or fewer) fertile stamens. Flowers of *S. hayesiana* (subclade IVb) display only the four middle stamens at anthesis, while they lack the adaxial staminodes and the abaxial stamens (Irwin and Barneby 1982; Marazzi, personal observation). Staminodes and highly reduced abaxial stamens are present, but fall off before anthesis. Anthers of the lateral abaxial stamens have two apical slits (*S. skinneri*, fig. 3A, species of subclade IVa, fig. 3B, species of subclade IVb: *S. chloroclada* [accession *35285*], *S.*

macranthera var. *nervosa*, fig. 3C, *S. pinheiroi*, *S. rizzinii*), or the two slits are confluent into a more or less U-shaped pore (other species of subclade IVb, *S. chloroclada* [accession *BM128*], fig. 3D, *S. dariensis* var. *hypoglauca*, *S. mucronifera*, fig. 3E, *S. obtusifolia*, *S. pilifera*, *S. quinquangulata*, fig. 3F, *S. rugosa*). The pores or slits point inwards (*S. skinneri*, fig. 3A, species of subclade IVa, fig. 3B), or the anther tip is bent (subclade IVb), and the pores point thus more or less upwards (*S. mucronifera*, fig. 3E, *S. quinquangulata*, fig. 3F), or outwards (*S. macranthera* var. *nervosa*, fig. 3C). The lateral furrow is continuous (species of subclade IVa, fig. 3B, and *S. macranthera* var. *nervosa* of subclade IVb), not continuous (*S. skinneri*, other species of subclade IVb: *S. chloroclada*, fig. 3D, *S. mucronifera*, fig. 3E, *S. obtusifolia*, *S. pilifera*), or absent (*S. quinquangulata*, subclade IVb, fig. 3F). The thecae are either not confluent, and pollen of each theca is released by the dehiscence slit of that theca (*S. skinneri*, fig. 4A), or they are apically confluent, forming a shared chamber (species of subclades IVa and IVb), and pollen traverses this chamber and is released either through the two separate slits (fig. 4B), or through two separate short canals opening in the U-shaped confluent pore (figs. 3E, 4C). The shared chamber may be absent in *S. quinquangulata*, because of lacking anther tip elongation (fig. 3F). A few species have unusually long tubular anther tips, almost up to 2.5 mm long (*S. mucronifera*, fig. 4Ci; *S. pilifera*). One layer of hypodermal cells adjacent to the stomium is specialized as described in clade I, and the walls of other hypodermal cells and several layers of subhypodermal cells are also strongly thickened (*S. skinneri*, fig. 4A, sections ii-iv, *S. mucronifera* of subclade IVb, fig. 4C), or only one layer of hypodermal cells is thick-walled throughout the anther wall (*S. aciphylla* of subclade IVa, fig. 4B). A pollen sac placentoid is present at least in *S. skinneri* (fig. 4A, section vii) and apparently in *S. mucronifera* (fig. 4C, section vii). The vascular bundle extends almost up to the sterile anther tip (fig. 4A, sections iv, v; fig. 4B, sections iii, iv; fig. 4C, sections iv, v).

CLADE V – Anthers of the abaxial stamens have two apical slits that point inwards (figs. 3H, G). The lateral furrow is continuous (figs. 3H, G). The thecae are not confluent in *S. cana* var. *calva*, and the pollen of each theca is released by the dehiscence slit of that theca (fig. 4D). The pattern of wall thickenings of the hypodermal and subhypodermal cells in *S. cana* var. *calva* (fig. 4D, section iii-v) is as described for *S. skinneri* in clade IV (fig. 4A, sections ii-iv). A pollen sac placentoid is present in *S. cana* var. *calva* (fig. 4D, section vi). The vascular bundle extends up to the level of the dehiscence slits (fig. 4D, sections iii-iv).

CLADE VI – Anthers of the lateral abaxial stamens have two apical slits or pores (*S. acuruensis var. acuruensis*, fig. 5A, *S. aphylla*, *S. chacoënsis*, fig. 5B, *S. crassiramea*, *S. multijuga* var. *lindleyana* and var. *multijuga*, fig. 5C, *S. rigida*, fig. 5D, *S. williamsii*, fig. 5E), or the two slits are confluent into a single U-shaped pore (*S. acuruensis* var. *catingae*, fig. 5F, *S. aversiflora*, fig. 5G, *S. holwayana*, *S. pallida*, *S. robiniifolia*, *S. tonduzii*, fig. 5H). The lateral furrow is continuous (*S. acuruensis* var. *acuruensis*, fig. 5A, *S. williamsii*, fig. 5E), or is not continuous in the other 12 taxa studied of clade VI (figs. 5 B-D, F-H). The thecae are apically confluent, forming a shared chamber. Pollen traverses this chamber and is released either through more or less short canals ending into the separate slits (fig. 6A), or through the single U-shaped pore (fig. 6B). Most species of clade VI have unusually long tubular anther tips, almost up to 2-4 mm long (*S. acuruensis*, *S. aversiflora*, *S. holwayana*, *S. multijuga*, *S. pallida*, *S. tonduzii*. *S. williamsii*, see figs. 5-A, C, E-H). In *S. tonduzii* (fig. 6B, sections ii-v), the pattern of cell wall thickenings of the hypodermal and other subhypodermal cells is as described for *S. skinneri* (clade IV, fig. 4A, sections ii-iv) and for *S. cana* var. *calva* (clade V, fig. 4D, section iii-v). In contrast, anther tips of *S. chacoënsis*, lacking the specialized hypodermal cells adjacent to the stomium described for clade I, have hypodermal and a few layers of subhypodermal cells that appear uniformly and strongly thick-walled (fig. 6B, sections ii-vi). In the anther, below the elongated tip, there is only one layer of such thick-walled cells (fig. 6B, sections vii, viii). A pollen sac placentoid is present at least in *S. tonduzii* (fig. 6B, section vii). The vascular bundle either extends almost to the shared chamber (fig. 6A, sections vi, vii), or within part of the sterile anther tip (fig. 6B, sections iii, iv).

CLADE VII – Clade VII is divided into subclades VIIa and VIIb, which differ in androecial structure. In subclade VIIa, the six to seven fertile stamens are differentiated into a middle and an abaxial set, with the four middle stamens notably shorter than the abaxial ones, whereas in subclade VIIb all fertile stamens have more or less the same length and shape (Marazzi et al. 2006). Anthers of the lateral abaxial stamens have two apical pores (species of subclade VIIa: *S. barclayana*, *S. cernua*, fig. 5I, *S. hirsuta*, *S. occidentalis* [see also Venkatesh 1957], *S. purpusii*) or a U- or V-shaped pore by confluence of two pores (species of subclade VIIa: *S. birostris* var. *hookeriana*, *S. corymbosa*, *S. hilariana*, *S. morongii*, *S. pendula*, fig. 5J, *S. septemptrionalis*; species of subclade VIIb, figs. 5K, L). The lateral furrow is present only along the sides and is not continuous with the pores (see figs. 5I-L). The thecae are either not confluent (species of subclade VIIa), and pollen of each theca is released by the dehiscence pore of that theca (figs. 5I,6C), or by the U-shaped single pore by confluence (fig. 5J), or the thecae are apically confluent, forming a shared chamber (species of subclade VIIb, figs. 5K-L, 6D). Thick-walled specialized hypodermal cells, as described in clade I, surround the dehiscent pores, and, together with strongly thick-walled hypodermal cells, form an evenly thick layer throughout the anther, which may be more (fig. 6C) or less (fig. 6D) conspicuous. A pollen sac placentoid is present at least in *S. cernua* (fig. 6C, section vi). The vascular bundle extends almost to the sterile anther tip (fig. 6C, sections iv, v) or to the shared chamber (fig.6D, sections v, vi).

UNCERTAIN SPECIES – The androecium of *S. chrysocarpa* has seven stamens, in which the abaxial stamens are slightly larger than the middle ones, and three (adaxial) staminodes. Anther tips of the abaxial stamens in *S. chrysocarpa* are similar to those of species in subclade IVb and are characterized by two dehiscence pores, pointing more or less outwards, with the lateral furrow apparently not connected to the pores. The androecium of *S. hebecarpa* also has seven fertile stamens, but the abaxial ones are conspicuously longer than the middle ones. Anther tips of abaxial stamens in *S. hebecarpa* are similar to those of species of subclade VIIb (especially *S. purpusii*), with two dehiscence pores, pointing inwards, and the thecae apically not confluent. In contrast, *S. surattensis* has all stamens fertile, as typical of species of subclade IVa (*Senna* sect. *Psilorhegma*). The anther tips of abaxial stamens in *S. surattensis* are in fact similar to those of other studied species of subclade IVa (e.g., *S. aciphylla*, fig. 3B, 4B).

Anther dehiscence pattern of middle stamens

The two posterior middle stamens belong to the inner androecial whorl, and the two anterior middle stamens to the outer whorl. These four stamens are similar in shape and size, and the anther tip is usually not elongated, in contrast to the abaxial stamens (see previous section), and the thecae are not confluent in most species.

CLADE I – Anthers have two apical slits (*S. polyantha*, fig. 7A, *S. silvestris* var. *guaranitica*), or a single pore by confluence (*S. siamea*, fig.7B). The anther tip is bent in such a way that the pores point more or less outwards. The lateral furrow is continuous (*S. polyantha*, fig. 7A), not continuous (*S. silvestris* var. *guaranitica*), or absent (*S. siamea*, fig.7B).

CLADE II – Anthers have two apical pores (see figs. 7C-E). The pores point inwards (*S. italica*, fig. E), or the anther tip is bent in such a way that the pores point either more or less upwards (*S. nicaraguensis*, fig. C) or outwards (*S. paradictyon*, fig. D). The lateral furrow is continuous. In *S. paradictyon* the anther tip is elongated, and the thecae may thus be confluent and form a shared chamber, as in the abaxial stamens (see above).

CLADE III – Anthers have two apical pores (see figs. 7F, G). The pores point outwards. The lateral furrow is continuous.

CLADE IV – Anthers have two apical pores (species of subclade IVa, species of subclade IVb: *S. dariensis*, *S. macranthera* var. *nervosa*, fig. 7H, *S. mucronifera*, fig. 7I), or a single pore by confluence of two (*S. skinneri*, fig. 7J). The lateral furrow is continuous (species of subclade IVa) or not continuous (*S. skinneri*, fig. 7J, species of subclade IVb, see figs. 7H,I). In species of

subclade IVa, anthers of the middle stamens are similar to those of the abaxial stamens, but slightly smaller.

CLADE V – Anthers have two apical pores (see fig. 7K). The pores point more or less upwards or outwards. The lateral furrow is continuous.

CLADE VI – Anthers have two apical pores (*S. acuruensis* var. *acuruensis*, *S. aphylla*, *S. chacoënsis*, *S. crassiramea*, *S. holwayana*, *S. multijuga* var. *lindleyana* and *var. multijuga*, fig. 7L, *S. rigida*, *S. robiniifolia* and *S. williamsii*), or a single, more or less U-shaped pore by confluence of two (*S. acuruensis* var. *catingae*, *S. aversiflora*, fig. 7M, *S. pallida*, *S. tonduzii*, fig. 7N). The pores point outwards. The lateral furrow is continuous (*S. crassiramea*, *S. holwayana*, *S. pallida*) or not continuous (see e.g., *S. multijuga* var. *multijuga*, fig. 7L, *S. tonduzii*, fig. 7N).

CLADE VII – Anthers have two apical pores (species of subclade VIIa: *S. barclayana*, *S. cernua*, *S. corymbosa*, *S. hirsuta*, fig. 7O, *S. occidentalis*, *S. purpusii*), or a U- or V-shaped single pore by confluence of two (species of subclade VIIa: *S. birostris* var. *hookeriana*, *S. hilariana*, *S. morongii*, *S. pendula*, fig. 7P, *S. septemtrionalis*; species of subclade VIIb). The lateral furrow runs along the side only and is not connected to the pore (see figs. 7O, P), except in *S. corymbosa*. The thecae are confluent in subclade VIIb, forming a shared chamber as in the abaxial stamens (see above).

UNCERTAIN SPECIES – Anther tips of *S. chrysocarpa* are similar to anther tips of species of subclade IVb (especially to *S. macranthera* var. *nervosa*), with two dehiscence pores, pointing outwards, and the lateral furrow not connected to the pores but only along the sides of the thecae . Anthers tips in *S. hebecarpa* are similar to those of species of subclade VIIb (especially to *S. purpusii*), with two dehiscence pores, pointing outwards. Anther tips in *S. surattensis* are similar to those of other species studied of subclade IVa, with two dehiscence pores.

Staminodes

In most *Senna* species, except for sect. *Psilorhegma*, the three adaxial stamens are sterile (i.e. do not produce pollen), and in some species of subclade VIIa, the median abaxial stamen is also sterile. This median staminode is of different form than the other staminodes. The two (adaxial) lateral staminodes belong to the outer androecial whorl, whereas the median one belongs to the inner whorl. These staminodes have rudimentary, flattened anthers (fig. 8). In the two lateral staminodes, anthers may be asymmetric, since their two thecae are of different length (e.g., figs. 8A, B, D, E, F, G, J, N), but the median staminode is usually symmetric (e.g., figs. 8C, H, I, K, L, M, O, R). The staminodes may be arrow-like (e.g., figs. 8C, H, O), or spatulate to petaloid (e.g., figs. 8F, G, I, S), or more or less strongly twisted (figs. 8E, Q), or highly reduced (e.g., figs. 8P, T). The lateral furrow is more or less conspicuous.

Development of filament union and lateral furrow

In *S. tonduzii* the stamens are united (fig. 9); this feature is here reported for the first time in the genus *Senna*. The filaments of the (adaxial) staminodes are united, and the left and right lateral staminodes are congenitally united with the filaments of the left and the right middle stamens, respectively. These latter ones are also united pairwise with each other. Filament union extends almost up to the attachment of the anthers. The organs appear still free in young buds (fig. 9A). Union originates by zonal growth of the common base of the organs, first appearing between lateral staminodes and middle stamens of the inner androecial whorl (fig. 9B), then also between the other organs (fig. 9C). In contrast, the abaxial stamens remain free (fig. 9D).

In many species, the lateral furrow in mature stamens is not connected to the dehiscence pores (see above). We studied the development of abaxial stamens in one such species, *S. mucronifera* of clade IVb, to explore the extension of the lateral furrow before anther tip elongation. When the anther tip starts to elongate, the lateral furrow - in form of a weak

depression - still extends along the entire length of the thecae (fig. 9E). This weak lateral depression disappears along the sterile anther tip during the elongation of the anther tip, whereas it remains along the sides of the thecae, becoming deeper, until the mature lateral furrow is formed (fig. 9F).

Stigma

The stigmatic surface in *Senna* is either enclosed in a chamber (i.e. chambered stigma; species of clades II-VII; figs. 10C-G, I-L, 11B-I, L) or it is exposed (i.e. crater-like stigma; species of clades I, IVa, V, VIIa; figs. 10A, B, H, 11J). Chambered stigmas point usually inwards (figs. 10 C-G, I-L, 11C-I, K; *S. armata* of subclade VIIb), or rarely upwards (*S. cana* var. *calva*, fig. 11B). Crater-like stigmas also point either inwards (figs. 10A,B, H), or upwards (figs. 11A, L). In general, the stigmatic orifice may be more or less punctiform (i.e. almost closed; figs. 10E, F, J), circular (figs. 10C, H, K, L, 11A, E, F, H, I, L), elliptic (figs. 10A, B, G, 11B-D, G, J, K), or slit-like (fig. 10I). The diameter of the orifice of chambered stigmas varies from almost zero (*S. mollissima*, fig. 10F) to approximately 1,4 mm (*S. quinquangulata*; measured on fig. 10K). One layer of hairs (species of clades IV-VII; figs. 10G, H, J-L, 11A-H, K) or several layers (species of clades I, VIIa; figs. 10A, B, 11J) may fringe the stigmatic orifice, or hairs are absent (species of clades II, III, IVa; figs. 10C-F, I, 11I, L). Secretion is especially visible and copious in the SEM samples of species with a crater-like stigma and several layers of fringing hairs (figs. 10A, B, 11J), but is more or less obvious also in other crater-like stigmas (figs. 10H, 11A), and in chambered stigmas (figs. 10E, J, 11B, C, E, F, H, K, L). Secretion may be produced also from the stigma of *S. macranthera* var. *nervosa*, since a large amount of pollen grain seems to stick on its stigmatic orifice (fig. 10L), and even from stigmas of species whose SEM samples apparently lack the secretion (figs. 10C, G, I-K, 11D,G, I), since it might have been washed out during the preparation of the samples.

DISCUSSION

Diversity of the stamens

Androecia of buzz-pollinated flowers are characterized by different types and degrees of structural specialization associated with the unusual pollination mode (see Buchmann 1983). The androecium of *Senna* is probably one of the best examples to illustrate this specialization. Although only few species of Cassiinae were studied previously, *Senna* has been regarded as the genus with the greatest androecial diversity among Cassiinae (Venkatesh 1957; Lasseigne 1979). Our results support and extend this view. We found that diversity in the androecium of *Senna* species pertains to: (1) patterns of heteranthery (including anther fertility and size difference among sets of fertile stamens); (2) dehiscence patterns (i.e. two pores or slits, or one pore by confluence of two; presence or absence of a shared chamber and separate canals; and extension of the vascular bundle); (3) pointing direction of the pores (i.e. inwards, upwards, or outwards); (4) extension of the lateral furrow (i.e. from the ends of the dehiscent pores along the entire thecae, or only along the sides of the thecae), (5) cell wall thickening patterns in the anther tip; and (6) stamen union. We also observed diversity in the form of anther base, but since it is not directly related to anther dehiscence, we do not further discuss it here.

Heteranthery in *Senna*, with up to four kinds of stamens, has fascinated researchers for a long time (e.g., Burk 1906). We investigated the variation observed in fertility vs. sterility (i.e. no pollen production), presence or absence of organs, and size difference between middle and abaxial anthers. We found seven patterns (fig. 12, diagrams i-vii): (i) all stamens fertile (subclade IVa; in some species one lateral abaxial stamen may be larger than the other ones); (ii) seven fertile stamens and three adaxial staminodes (species of clades I-VII), in which all abaxial stamens are longer than the middle stamens and their anthers are of similar size (clades IIIa, VI, VIIa), (iii) the median abaxial stamen is smaller than the lateral ones, but still longer than the middle ones (clades I, II), (iv) the abaxial and the middle stamens are of similar size (clades IIIb, IVb, VIIb);

(v) only six fertile stamens, in which the abaxial stamens are longer than the middle ones, and three adaxial and the median abaxial organs staminodial (clade VIIa); (vi) similar to pattern v, but the median abaxial organ is absent (clades VIIa); (vii) only four middle stamens are fertile, and six organs are staminodial, falling off at anthesis (*S. hayesiana*, subclade IVb). It should be noted that in pattern ii of clade VI, although the anthers of the abaxial stamens are of similar size, their filaments may be of different lengths, and the stamens can thus have different sizes.

Anthers display diverse dehiscence patterns. They may have two dehiscent slits or pores, or a single pore by confluence of two, which is more or less V- or U-shaped. The anther tip (i.e. upper portion of anthers, above the pollen sacs) may be elongated, especially in abaxial stamens, and the thecae may be apically confluent, forming a shared chamber. The pollen thus traverses the chamber and is released by passing through two separate canals and then the pores, or directly either by the two separate pores or the single pore by confluence of two. The shared chamber and the canals are formed by decay of the tissue in the sterile anther tip, called resorption tissue by Venkatesh (1957). The vascular bundle more or less extends into the sterile anther tip. According to the number of pores or slits, presence or absence of a shared chamber and of separate short canals, and the extension of the vascular bundle, we found six patterns in *Senna* (fig. 12, diagrams A-F): (A) two separate pores, no shared chamber, vascular bundle almost or up to the anther tip; (B) two separate pores, shared chamber and separate canals present, vascular bundle almost to the chamber; (C) two separate pores, shared chamber present, but canals absent, vascular bundle almost to the chamber; (D) a single pore by confluence of two, no shared chamber, vascular bundle almost to the single pore; (E) a single pore by confluence, shared chamber and separate canals present, vascular bundle almost to the single pore; and (F) a pore by confluence of two, shared chamber present, but canals absent; vascular bundle almost to or into part of the chamber.

The morphologically least differentiated pattern A (two separate pores and separate thecae) is present at least partially in almost all clades except clades II and VI (fig. 12). Pattern D, with one pore by confluence and separate thecae, is restricted to a few species in clades I, IV and VII. Clades with even more specialized patterns, characterized by a shared chamber (gray boxes in fig. 12), are in some cases sister to clades characterized by the least differentiated pattern A. Patterns B and E, with canals present, may be considered as morphologically intermediate between the least differentiated patterns A and D and the most specialized C and F.

Among the sets of fertile stamens, the abaxial stamens display all six patterns, whereas the middle ones, likely because of no anther tip elongation, apparently display mostly patterns A and D, which lack the shared chamber. Exceptions are species of subclade VIIb, in which all anthers display pattern F, and possibly certain species of clade IVb, where microtome sections by Venkatesh (1957) show that middle stamens of *S. obtusifolia* have a shared chamber, but no anther tip elongation. The unusual tubular anther tips, up to several millimeters long in some species of clades IVb and, especially, VI, all have a shared chamber (patterns B, C, E, F), and most have a single pore by confluence (patterns E, F). Therefore, apical confluence of the thecae into a shared chamber seems to be a precondition for the tubular anther tip elongation. In addition to anther dehiscence, middle and abaxial stamens may also differ in size. We found the following patterns: the abaxial stamens are much longer and more arched than the middle stamens and have (*i*) a different pattern of anther dehiscence, usually corresponding to one of the specialized patterns B, C, E, or F (clades II, IVb, VI and VIIa; fig. 12), or, (*ii*) more rarely, the same pattern of dehiscence (clades I, IIIa); or (*iii*) middle and abaxial stamens are of approximately the same size and share the same dehiscence pattern, which often corresponds to the less differentiated patterns A and D (subclades IIIb, IVa, IVb; fig. 12).

We found three more or less distinct pointing directions of the pores (fig. 12, diagrams a-c): (a) inwards (pores on the ventral anther side); (b) upwards (pores on the anther top); and (c) outwards (pores on the dorsal anther side). According to these patterns, pores pointing inwards are directed towards the flower center, whereas pores pointing upwards or outwards are directed away

from the flower center. Therefore, the pointing direction of the pores directly affects the direction of pollen release during buzzing.

Three different types of lateral furrows can be identified in the anthers (fig. 12, diagrams 1-3): (1) a continuous lateral furrow, i.e. the lateral furrow running from the lower end of each dehiscence slit or pore along the entire length of the theca (species of clades I-VI); (2) a non-continuous lateral furrow, i.e. present only along the sides of the thecae between the pollen sacs (species of clades I, II, IV, VI, VII); or (3) no lateral furrow, i.e. with the anther sides smooth (a few species of clades I, IV). Evidence from our developmental studies on *S. mucronifera* (figs. 9E, F), indicates that a non-continuous lateral furrow (pattern 2) appears to have the same early developmental pathway as the continuous lateral furrow (pattern 1). At the beginning of the anther tip elongation in young bud, the lateral furrow appears as a weak depression, running from the end of the pore along the entire length of the thecae, resembling an early stage of pattern 1 (fig. 9E). This weak depression successively disappears in the part of the elongating sterile anther tip, while it develops further along the sides of the thecae (fig. 9F).

As in many other buzz-pollinated angiosperms (Endress 1994, 1996), the endothecium is lacking, while hypodermal and subhypodermal cells are strongly thick-walled. Venkatesh (1957) first described anther cell wall thickenings and the role of such cells during dehiscence. He recognized two kinds of thick-walled cells: a specialized kind of subhypodermal radially elongated cells, usually adjacent to the region of the stomium, and a non-specialized kind of hypodermal and also subhypodermal cells in other anther parts. Although the present study does not focus on thickening patterns, we observed a trend between the size of the anthers of abaxial stamens and the thickening pattern of anther walls. In large and stiff anthers, there are several layers of strongly thick-walled hypodermal and subhypodermal cells continuous all around the anther wall (e.g., *S. cana* var. *calva*, fig. 4D, *S. mucronifera*, fig. 4C, *S. nicaraguensis*, fig. 2C, *S. skinneri*, fig. 4A, and *S. tonduzii*, fig. 6B). In contrast, in less stiff anthers (e.g., *S. paradictyon*, fig. 2B), or in small anthers (e.g., *S. aciphylla*, fig. 4B, *S. apiculata*, fig. 6D, *S. chacoënsis*, fig. 6A, and *S. polyantha*, fig. 2A), there is only one layer of thick-walled hypodermal cells continuous all around the anther.

Reported here for the first time in *Senna*, filament union is a unique feature of *S. tonduzii* (figs. 9A-D), and is rare in caesalpinioids. The filaments of the three staminodes and of the four stamens are united almost up to the attachment of the anthers, whereas the abaxial stamens are free (fig. 12, diagram vii). United filaments occur also in flowers of species of the distantly related caesalpinioid genera *Amherstia*, *Barnebydendron*, *Brownea*, *Eperua*, *Eurypetalum*, *Stemonocoleus*, *Baikiaea*, *Tessmannia*, *Sindoropsis*, *Sindora*, and *Tamarindus* (Endress 1994, Tucker 2000, Fougère-Danezan 2005; Detarieae s.l.-clade, e.g., Wojciechowski et al. 2004). However, in these and especially in several papilionoids, filament union involves abaxial organs, while the adaxial median organ is free. Therefore, the pattern of filament union observed in *S. tonduzii* is unique in the family. Congenital union of the filaments is unusual in buzz-pollinated flowers, whereas postgenital union of the anthers is more common, for example to form a tube as in *Lycopersicon* (see Endress 1994).

Diversity of the stigma

Stigmas of buzz-pollinated flowers are often point-tipped (e.g. Endress 1994). The diversity observed in point-tipped stigmas of *Senna* species prompted Owens and Lewis (1989) to divide them into five groups. These groups were not congruent with the sectional boundaries of the traditional classification of *Senna* by Irwin and Barneby (1982), and were not considered in subsequent studies (Dulberger et al. 1994). Adding our results, diversity in the stigmas of *Senna* species includes: presence or absence of a chamber (i.e. chambered vs. crater-like stigma), orifice position, form, size, and structure, and presence or absence of a secretion (figs. 10, 11). We did not find other kinds of stigmas, in addition to the chambered and crater-like ones, but we observed

a higher diversity in the position or pointing directions of the stigmas than previously recognized (Owens and Lewis 1989): inwards in most chambered and several crater-like stigmas, and upwards in several crater-like, but only in one chambered stigma.

Implication for systematics of Senna

Molecular phylogenetic analyses of *Senna* (Marazzi et al. 2006) did not support the traditional classification system of *Senna* based on Irwin and Barneby (1982), because most sectional boundaries (except for the monophyletic sect. *Psilorhegma*) were not congruent with the seven major clades and a number of subclades of the molecular phylogeny. Our study on anther tips, in particular of abaxial (pollinating) stamens, provides support for several of the clades and subclades of *Senna* (Marazzi et al. 2006). Anther tip morphology is constant in clades III and V (combination of patterns A1) and subclades VIIb and IVa (patterns F2b and C1a, respectively), whereas the remaining clades I, II, IV, VI, VII have diverse anther dehiscence patterns (see fig. 12). The results of the morphological analyses presented here support most of the traditional series (Irwin and Barneby 1982) that are monophyletic in Marazzi et al. (2006) (see black longitudinal bars in fig. 12, from top). Subclade VIIa*, equivalent to ser. *Coluteoideae* but including also *S. birostris* of ser. *Stipulaceae*, is characterized by pattern D2; subclade VIIa**, equivalent to ser. *Basiglandulosae*, is characterized by A2; subclade VIa, equivalent to ser. *Aphyllae*, is characterized by C2b, lacking the long tubular anther tips of the other species of clade VI; subclade IVb*, equivalent to ser. *Trigonelloideae*, is characterized by E2; subclade IIIa, equivalent to ser. *Deserticolae*, is characterized by abaxial stamens longer than the middle ones; in contrast, subclade IIIb, equivalent to ser. *Isandrae*, is characterized by abaxial stamens as long as the middle ones.

Based on the combination of anther traits described in this study, we identified *S. chrysocarpa*, *S. hebecarpa* and *S. surattensis*, the three additional species not considered in the molecular phylogeny of *Senna* (Marazzi et al. 2006), as probable members of subclade IVb, clade VII and subclade IVa, respectively. Although molecular phylogenetic analyses are necessary to clarify the position of these species within these clades, anther traits proved to be taxonomically useful, as previously suggested (Venkatesh 1957, Lasseigne 1979; Irwin and Barneby 1982).

In most cases where multiple accessions were investigated, it was possible to conclude that patterns of anther dehiscence appeared to be consistent within species. However, in some species, especially those characterized by broad morphological variation and, hence, subdivided in multiple varieties by previous taxonomists, patterns of anther dehiscence were variable. For example, our structural results are consistent with the paraphyly of the *S. acuruensis* complex, as suggested by Marazzi et al. (2006). Abaxial stamens of *S. acuruensis* var. *acuruensis* are more similar to those of the sister species *S. multijuga*, with two dehiscence pores, than to those of *S. acuruensis* var. *catingae*, with a single U-shaped pore by confluence of two (figs. 5A, C, F). *Senna birostris* includes seven varieties (Irwin and Barneby 1982, Laporta et al. 2006). According to recent taxonomic work (Laporta et al. 2006), *S. birostris* var. *hookeriana* is distinguished from the newly described var. *jallaguensis* based on the number of anther pores: two in var. *hookeriana*, and a single pore in var. *jallaguensis*. Another species with different anther dehiscence patterns is *S. chloroclada* (subclade IVb): abaxial anthers of our Paraguayan accession have two apical slits, whereas those of the Argentinean accession have a single U-shaped pore by confluence (fig. 3D). However, unlike *S. acuruensis* and *S. birostris*, in *S. chloroclada*, these different patterns of anther dehiscence are not ascribed to different varieties.

Evolutionary aspects of the androecium

Traditional interpretations of evolutionary trends in the floral morphology of *Senna* considered the fertility of all stamens (pattern i; fig. 12), typical of *Senna* section *Psilorhegma*, to be the ancestral condition within the genus (Irwin and Barneby 1982; Randell 1988). Molecular phylogenetic

studies, however, did not support this hypothesis (Marazzi et al. 2006). In fact, the position of section *Psilorhegma*, embedded in clade IV, suggested that fertility of all stamens is a synapomorphy of subclade IVa, and likely represents a derived condition in *Senna*. Conversely, an androecium formed by seven or fewer fertile stamens and three staminodes, a condition found in all remaining clades (patterns ii-vii; fig. 12), probably characterized the ancestral *Senna* flower. Character optimization studies are necessary to evaluate which of the patterns ii-vii may represent the ancestral condition of the androecium.

Anthers with a single pore were traditionally considered to be derived from anthers with two pores in *Senna* (Irwin and Barneby 1982; Randell 1988). Anthers with two pores (fig. 12, patterns A-C) occur in all species of clades II and III, and in many species of the other major clades (I, IV-VII), whereas anthers with a single pore occur in some species of unrelated clades I, IVb, VI, VIIa*, VIIb. However, anthers with two pores or a single pore do not necessarily represent the same pattern of anther dehiscence: two pores occur in patterns A-C, while a single pore occurs in patterns D-F (fig. 12). These patterns are distinguished from one another by other features, such as presence or absence of a shared chamber and short canals, in addition to the number of anther pores. This means that, for example, anthers with two pores of pattern A may not be homologous to anthers with two pores of pattern C. Therefore, structural differences among anther dehiscence patterns should be considered when reconstructing the evolution of the number of anther pores within a phylogenetic framework.

Implications for pollination biology and floral evolution

Several structural specializations of *Senna* flowers, including different kinds of stamens (middle feeding stamens vs. abaxial pollinating stamens), diverse pointing directions of the dehiscence pores, and different kinds of point-tipped stigmas, probably evolved in relation to buzz pollination. Our observation in *Senna* that anther tips of abaxial stamens are more diverse and more specialized than those of the middle stamens may be related to a separation of function between feeding anthers (with pollen used as food for bee larvae), and pollinating anthers (with pollen used for plant reproduction). The separation of function may in turn be related to the intra-floral behaviour of the pollen collecting bees. In particular, the positioning of the bees on the androecium may have influenced the evolution of anther morphology and of diverse strategies of pollen release.

Although little is known about the details of pollination biology in *Senna*, studies on a few species have shown that in order to vibrate the flowers, bees usually clasp with their legs the middle stamens only, which then eject the pollen towards the ventral side or the legs of the bees, while the abaxial anthers eject the pollen in other directions, such as towards the flanks or the dorsal side of the bees (e.g. Delgado Salinas and Souza Sanchéz 1977; Gottsberger and Silberbauer-Gottsberger 1988, Westerkamp 2004). This situation is found in flowers that have middle and abaxial stamens of different sizes, where the bees clasp only the shorter anthers of the middle stamens and not the anthers of the much longer and often arched abaxial stamens. These abaxial stamens show the greatest anther tip diversity in *Senna*. In flowers with stamens of the same size, anther morphology is similar and usually of the least differentiated patterns A and D (fig. 12), and pollen release is always on the ventral side or on the legs of the bees. All anthers appear thus to function as feeding anthers. In these flowers, the bees may not only clasp the anthers of the middle stamens but also those of the abaxial stamens. Therefore, the clasping of anthers by the bees seems to have favoured the evolution of rather unspecialized anther dehiscence patterns, releasing pollen to the ventral side and/or legs of the bees, where anther tip elongation is thus probably useless. In contrast, the abaxial stamens, which are not clasped by the bees, have undergone a remarkable divergent evolution of anther tip specializations. Of particular interest are the different pointing directions of their pores (i.e. inwards, upwards, outwards; fig. 12, diagrams 1-3) and types of anther tip elongation, with important implications for pollen discharge and dispersal.

The orientation of the pores directly affects the direction of pollen release. Pores pointing inwards likely release pollen towards the flower centre, and thus towards the body of the bees. In this case, the morphology of the anthers would allow control of the direction (downwards or forwards) of the ejected pollen flow. In contrast, pores pointing upwards or outwards likely release the pollen in the opposite direction. In this case, we expect the pollen to be ejected towards the petals, which should be positioned to reduce loss of pollen from the flower. The pollen flow is thus ricocheted by the petals towards the body of the bees (e.g. Delgado Salinas and Souza Sánchez 1977; Westerkamp 2004).

Tubularly elongated sterile anther tips are rare in angiosperms, but occur in numerous taxa with buzz-pollinated flowers. A tubular anther tip structure ensures a precise direction of pollen ejection, since only those pollen grains moving exactly in the direction of the tube will be released by the anthers (Buchmann and Hurley 1978). To our knowledge, in addition to *Senna*, tubular anther tips are present in four families, including Elaeocarpaceae/Tremandraceae (e.g., in *Platytheca galioides* and *Tetratheca thymifolia*, Matthews and Endress 2002), Ericaceae (e.g., in *Demosthenesia cordifolia*, Luteyn 1978; *Diplycosia* spp., Sleumer 1967; *Vaccinium* spp., Sleumer 1967; Danet 2005), Melastomataceae (e.g. *Tococoa* spp., Michelangeli 2006), and Solanaceae (e.g. *Solanum mortonii*, Hunziker 2001; *Solanum quitoense*, B.M. personal observation; and *Solanum rostratum*, Todd 1882). The most specialized anther tips in *Senna* have apically confluent thecae, which form a shared chamber and may be variably elongated (up to several millimeters long) or constricted at their base (close to the fertile thecae). However, diverse kinds of elongation and constriction may also reflect different strategies of pollen discharge, which influence (1) the speed of the pollen flow ejected and/or (2) the amount of pollen released by the vibrations of the bees.

In the first case, anther tip elongation may funnel the pollen flow, causing it to be accelerated and ejected at a higher speed. This may be seen by blowing into the wider side of a horn: the air flow is compressed towards the narrower side of the horn and will thus be released with an increased speed. The decreasing lumen towards the narrower end causes compression of the pollen flow. An increase in the speed of the pollen flow would then allow the pollen to have enough speed to ricochet against the petals, (which vibrate and contribute to rebound the pollen), finally adhering to the body of the bees. Flowers with long and tubular anthers with upwards and/or outwards pointing pores have strongly concave lower petals, which are often also highly modified in both shape and size. The corolla of these flowers is thus highly asymmetric. Work in progress will test whether there is a correlation between the strong petal modification and specialized patterns of anther dehiscence.

In the second case, the constriction between the thecae and the sterile anther tip in *Senna* may influence the amount of pollen released. The narrower the constriction, the smaller the amount of released pollen, a condition that favors the discharge of pollen in multiple, smaller packages and makes pollen available for successive pollinator visits. This mechanism, known as packaging, influences the amount of pollen presented on the anthers (Harder and Thomson 1989). Pollen packaging has been described, for example, in flowers of *Penstemon* s.l. (Scrophulariaceae; Castellanos et al. 2006). Anthers of bee-pollinated penstemons have narrower dehiscence openings and present their pollen more gradually than anthers of hummingbird-adapted relatives. Gradual pollen discharge should compensate the inefficiency of pollen delivery by frequent and wasteful pollinators, for ex., bees, as compared to more efficient pollinators, for ex., hummingbirds, which do not groom pollen from their body (Castellanos et al. 2003, 2006). However, experimental studies conducted on buzz-pollinated species (but not on *Senna* species) showed that pollen removal increased with flower age (e.g., Harder and Barclay 1994). In this mechanism, known as dispensing, pollen is gradually presented by restricting the amount of pollen removed from a package during a single visit (Harder and Thomson 1989).

Stiffness of anthers and filaments, and filament union in *S. tonduzii* are other features that appear to have evolved in relation to buzz-pollination, for they allow the bee to firmly clasp the

anthers and improve the transmission of the vibrations from its body to all stamens, thus causing pollen release. We observed that anther wall thickening appears to be correlated with flower size. Abaxial anthers of small flowers tend to have only one layer of thick-walled cells, whereas those of larger flowers tend to have several layers (see above). The relation between anther wall thickenings and flower size may be interpreted as a consequence of the need of anthers to be sufficiently stiff to act as a resonating chamber for pollen release by buzzing, and may be associated with particular features of the size of visiting bees and/or the intensity of vibrations. As Buchmann and Hurley (1978) showed that the intensity of vibrations increases with the size of the insect, we would expect bees of different sizes to be unequally efficient in pollen removal from the same flower. Measurements of pollen removal from *S. reticulata* showed that large bees removed more pollen in one visit (about 15-20% of total initial available pollen) than did smaller bees (Snow and Roubik 1987).

The role of the staminodes in buzz-pollination is enigmatic. Gottsberger and Silberbauer-Gottsberger (1988) observed that bees sometimes bite the staminodes to avoid dropping out of the flowers while buzzing. However, they may also play a role in pollinator attraction. Many *Senna* species display in fact staminodes with flat, enlarged and often yellow or brownish sterile anthers, e.g., in species of clades I, II, III, V, VI, and subclade VIIa. In contrast, the staminodes of other species they apparently lack such a function, for they are highly reduced and rather cryptic (subclade IVb, and some of clade VI).

Most of the diversity we observed in the stigma involves its shape (i.e. chambered or crater-like), the hairs fringing the stigmatic orifice, and presence or absence of secretion. The minute, point-tipped stigmas touch or approach such sites of the body of the bees where pollen is not groomed. Owens and Lewis (1989) suggested that the hairs fringing the stigmatic orifice may remove pollen from the bee's body, acting as a fine comb, while Dulberger et al. (1994) suggested that electrostatic forces may aid pollen capture and its adhesion to those stigmatic tips lacking hairs. However, while brushing and electrostatic forces may be instrumental for pollen transport to the stigma, they may not serve any special function to promote pollen movement into the stigmatic chamber, for which the vibrations of the bees appear to be necessary (Dulberger 1981; Dulberger et al. 1994; van Kleunen and Johnson 2005; Laporta 2003). Instead, the fringing hairs are probably a device for protecting the stigmatic secretion (Dulberger et al. 1994). Most stigmas are associated with a long and often strongly arched gynoecium, and the stylar tip may even be strongly inflected (Owens and Lewis 1989; Dulberger et al. 1994). We observed that most of these long gynoecia have a chambered and inwards-pointing, crater-like stigma. In contrast, upwards-pointing crater-like stigmas appear to be associated with short gynoecia of species that are common weeds. These weeds have small and probably autogamous flowers (e.g., *S. uniflora*, Irwin and Barneby 1982, clade V, fig. 11A, and the group of *S. apiculata*, *S. argentea* and *S. crotalarioides*, subclade VIIb). Dulberger et al. (1994) suggested that the diversity in inflection of the style tip and stigma position and orientation evolved in relation to the specific size and positioning of the bees visiting the flowers. Whether there is an additional correlation between the stigma type and breeding system has not yet been explored.

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TABLE 1

Taxa used in this study, source, and voucher information. Clades or subclades of *Senna* by Marazzi et al. (2006) and section by Irwin and Barneby (1982) are given in brackets (AS, *Astroites*; CH, *Chamaefistula*; PA, *Paradictyon*; PE, *Peiranisia*; PS, *Psilorhegma*; SE, *Senna*; unc., clade uncertain). Acronyms of herbaria or botanical gardens (in alphabetic order): CBG (=ANBG) = Australian National Botanic Gardens, BGB = Botanical Garden of the University of Basel, BGM = Botanischer Garten der Universität München, BGZ = Botanischer Garten der Universität Zürich, CAY = Institut de Recherche pour le Developpement (IRD), Cayenne, CTES = Instituto de Botánica del Nordeste, Corrientes, G = Conservatoire et Jardin Botaniques de la Ville de Genève, HUEFS = Universidad Estadual de Feira de Santana, MEXU = Universidad Nacional Autónoma de México, MT = Université de Montréal, PBIB = Parco Botanico Isole di Brissago, PMA = Universidad de Panamá, PTGB = National Tropical Botanical Garden, Kauai, PY = Museo Nacional de Historia Natural de Paraguay, RBGA = Royal Botanic Garden Mount Annan, SI = Instituto de Botánica Darwinion, San Isidro, STRI = Smithsonian Tropical Research Institute, Balboa, Z = University of Zurich and Botanical Garden.

Taxon	**Clade**	**Sect.**	**Source**	**Voucher nr.**	**Locality and herbaria**
S. aciphylla (Benth.) Randell	IVa	PS	Cult.	CGB 9900061	garden CBG, CBG, Z
S. aciphylla (Benth.) Randell	IVa	PS	Cult.	CGB 9900061	garden CBG, CBG, Z
S. acuruensis* var. *acuruensis (Benth.) H.S. Irwin & Barneby	VI	PE	Wild	*Queiroz & Marazzi LQ 9198*	Brazil, Bahia, HUEFS, Z
S. acuruensis* var. *catingae (Harms) H.S. Irwin & Barneby	VI	PE	Wild	*Queiroz & Marazzi LQ 9177*	Brazil, Bahia, HUEFS, Z
S. alata (L.) Roxb.	II	SE	Cult.	*Vanni & Marazzi 4516*	Argentina, Corrientes, CTES, G
S. alata (L.) Roxb.	II	SE	Wild	*Marazzi & al. BM026*	Paraguay, Caaguazú, PY, CTES, Z
S. andrieuxii (Benth.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM162*	Mexico, Puebla, MEXU, Z
S. aphylla (Cav.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM084*	Argentina, Santiago del Estero, CTES, Z
S. apiculata (M. Martens & Galeotti) H.S. Irwin & Barneby	VIIb	CH	Wild	Marazzi & Flores BM170	Mexico, Puebla, MEXU, Z
S. argentea (Kunth) H.S. Irwin & Barneby	VIIb	CH	Wild	*Marazzi & Flores BM175*	Mexico, Oaxaca, MEXU, Z
S. armata (S. Watson) H.S. Irwin & Barneby	VIIb	CH	Wild	*Schönenberger JS751*	USA, California, Z
S. artemisioides (DC.) Randell	IVa	PS	Cult.	*Marazzi BM002*	garden BGZ s.n., Zurich, Z
S. atomaria (L.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM173*	Mexico, Oaxaca, MEXU, Z
S. aversiflora (Herbert) H.S. Irwin & Barneby	VI	PE	Wild	*Queiroz & Marazzi LQ 9204*	Brazil, Bahia, HUEFS, Z
S. barclayana (Sweet) Randell	VIIa	CH	Cult.	*Marazzi BM136*	garden PBIB 2003/76, Z
S. birostris (Hook.) H.S. Irwin & Barneby (cf. var. *jallaguensis* based on Laporta et al. 2006)	VIIa	CH	Wild	*Marazzi et al. BM090*	Argentina, Tucumán, CTES, Z
S. cana* var. *calva H.S. Irwin & Barneby	V	CH	Wild	*Conceiçao & Marazzi 1132*	Brazil, Bahia, HUEFS, Z

S. cernua (Balb.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM007*	Paraguay, Caaguazú, PY, CTES, Z
S. chacoënsis (L. Bravo) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM083*	Argentina, Santiago del Estero, CTES, Z
S. chloroclada (Harms) H.S. Irwin & Barneby	IVb	PE	Wild	*Marazzi et al. BM128*	Argentina, Salta, CTES, Z
S. chloroclada (Harms) H.S. Irwin & Barneby	IVb	PE	Wild	*Schinini & Marazzi 35285*	Argentina, Formosa, CTES, G
S. chrysocarpa (Desv.) H.S.Irwin & Barneby	unc.	CH	Cult.	*Prévost* 4915	French Guyana, Cayenne, CAY, Z
S. corymbosa (Lam.) H.S. Irwin & Barneby	VIIa	CH	Cult.	*Marazzi et al. BM103*	Argentina, Tucumán, CTES, Z
S. crassiramea (Benth.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM120*	Argentina, Jujuy, CTES, Z
S. crotalarioides (Kunth) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi & Flores BM163*	Mexico, Puebla, MEXU, Z
S. dariensis* var. *hypoglauca H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi & Alvárez BM153*	Republic of Panama, Coclé, PMA, STRI, Z
S. didymobotrya (Fresen.) H.S. Irwin & Barneby	II	SE	Cult.	*Marazzi BM002*	garden BGZ 19700009, Z
S. galeottiana (M. Martens) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi & Flores BM165*	Mexico, Puebla, MEXU, Z
S. hayesiana (Britton & Rose) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi & Alvárez BM150*	Republic of Panama, Panamá, PMA, STRI, Z
S. hebecarpa (Fernald) H.S.Irwin & Barneby	unc.	CH	Cult.	s.n.	garden BGM, Z
S. hilariana (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM027*	Paraguay, Alto Paraná, PY, CTES, Z
S. hirsuta* var. *hirta (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM115*	Argentina, Salta, CTES, Z
S. hirsuta* var. *leptocarpa (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM065*	Paraguay, San Pedro, PY, CTES, Z
S. holwayana* var. *holwayana (Rose) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Flores BM161 & 166*	Mexico, Puebla, MEXU, Z
S.* indet. ser. *Bacillares	IVb	CH	Wild	*Marazzi & Alvárez BM160*	Republic of Panama, Panamá, PMA, STRI, Z
S. italica Mill.	II	SE	Wild	*Zietsmann 4345*	Republic of South Africa, Free State, NMB, Z
S. macranthera cf.* var. *nervosa (Vogel) H.S. Irwin & Barneby	IVb	CH	Cult.	*Vanni & Marazzi 4543*	Argentina, Misiones, CTES, G
S. macranthera* var. *nervosa (Vogel) H.S. Irwin & Barneby	IVb	CH	Cult.	*Marazzi et al. BM082*	Paraguay, Caaguazú, PY, CTES, Z
S. mollissima (Willd.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM181*	Mexico, Oaxaca, MEXU, Z
S. morongii (Britton) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM130*	Argentina, Salta, CTES, Z
S. morongii (Britton) H.S. Irwin & Barneby	VIIa	CH	Wild	*Schinini & Marazzi 35279*	Argentina, Chaco, CTES, G
S. mucronifera (Benth.) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi et al. BM019*	Paraguay, Caaguazú, PY, CTES, Z
S. multijuga* var. *lindleyana (Gardner) H.S. Irwin & Barneby	VI	PE	Wild	*Queiroz & Marazzi LP 9226*	Brazil, Bahia, HUEFS, Z
S. multijuga* var. *multijuga (Rich.) H.S. Irwin & Barneby	VI	PE	Cult.	*Marazzi & Alvárez BM151*	Republic of Panama, Panamá, PMA, STRI, Z
S. nicaraguensis (Benth.) H.S. Irwin & Barneby	II	SE	Wild	*Marazzi & Flores BM185*	Mexico, Chiapas, MEXU, Z
S. obtusifolia (L.) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi et al. BM024*	Paraguay, Caaguazú, PY, CTES, Z

S. obtusifolia (L.) H.S. Irwin & Barneby	IVb	CH	Wild	*Vanni & Marazzi 4514*	Argentina, Corrientes, CTES, G
S. occidentalis (L.) Link	VIIa	CH	Wild	*Marazzi et al. BM060*	Paraguay, Caaguazú, PY, CTES, Z
S. odorata (Morris) Randell	Iva	PS	Cult.	ANBG 68349	Australia, CBG
S. pallida (Vahl) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Flores BM178*	Mexico, Oaxaca, MEXU, Z
S. paradictyon (Vogel) H.S. Irwin & Barneby	II	PA	Wild	*Marazzi et al. BM028*	Paraguay, Alto Paraná, PY, CTES, Z
S. pendula (Willd.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM117*	Argentina, Salta, CTES, Z
S. pilifera **var. *pilifera*** (Vogel) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi et al. BM011*	Paraguay, Caaguazú, PY, CTES, Z
S. pilifera **var. *pilifera*** (Vogel) H.S. Irwin & Barneby	IVb	CH	Wild	*Vanni & Marazzi 4513*	Argentina, Corrientes, CTES, G
S. pinheiroi H.S. Irwin & Barneby	IVb	CH	Wild	*Queiroz 9210*	Brazil, Bahia, HUEFS.
S. polyantha (Collad.) H.S. Irwin & Barneby	I	CH	Wild	*Marazzi & Flores BM172*	Mexico, Oaxaca, MEXU, Z
S. purpusii (Brandegee) H.S. Irwin & Barneby	VII	CH	Cult.	*Marazzi BM004*	garden BGB 3585/96-P, Z
S. quinquangulata (Rich.) H.S. Irwin & Barneby	IVb	CH	Wild	*Queiroz & Marazzi LQ 9220*	Brazil, Bahia, HUEFS, Z
S. rigida (Hieron.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM108*	Argentina, Salta, CTES, Z
S. rizzinii H.S. Irwin & Barneby	IVb	CH	Wild	*Conceição & Marazzi* 1126	Brazil, Bahia, HUEFS, Z
S. robiniifolia (Benth.) H.S. Irwin & Barneby	VI	PE	Cult.	*Marazzi BM005*	garden BGM 98/3500w, Z
S. rugosa (G. Don) H.S. Irwin & Barneby	IVb	CH	Wild	*Queiroz & Marazzi LQ 9181*	Brazil, Bahia, HUEFS
S. septemtrionalis (Viviani) H.S. Irwin & Barneby	VIIa	CH	Cult.	*Marazzi BM140*	garden BGM s.n., Z
S. siamea (Lam.) H.S. Irwin & Barneby	I	CH	Cult.	*Marazzi & Alvárez BM157*	Panamá City, Panamá, PMA, STRI, Z
S. silvestris **var. *guaranitica*** (Chodat & Hassl.) H.S. Irwin & Barneby	I	CH	Wild	*Marazzi et al. BM068*	Paraguay, San Pedro, PY, CTES, Z
S. skinneri (Benth.) H.S. Irwin & Barneby	IV	CH	Wild	*Marazzi & Flores BM176*	Mexico, Oaxaca, MEXU, Z
S. spectabilis (DC.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi et al. BM029*	Paraguay, Alto Paraná, PY, CTES, Z
S. spectabilis **var. *spectabilis*** (DC.) H.S. Irwin & Barneby	III	PE	Wild	*Vanni & Marazzi 4530*	Argentina, Misiones, CTES, G
S. surattensis (Burm.f.) H.S.Irwin & Barneby	unc.	PS	Cult.	*Endress* 03-91	garden PTGB, USA, Hawaii, Z
S. tonduzii (Standl.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Flores BM187*	Mexico, Chiapas, MEXU, Z
S. uniflora (Mill.) H.S. Irwin & Barneby	V	CH	Wild	*Marazzi & Flores BM186*	Mexico, Chiapas, MEXU, Z
S. unijuga (Rose) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM167*	Mexico, Puebla, MEXU, Z
S. villosa (Mill.) H.S. Irwin & Barneby	VIIb	VS	Wild	*Marazzi & Flores BM174*	Mexico, Oaxaca, MEXU, Z
S. williamsii (Britton & Rose) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Alvárez BM158*	Republic of Panama, Panamá, PMA, STRI, Z
S. wislizeni (A. Gray) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM169*	Mexico, Puebla, MEXU, Z.

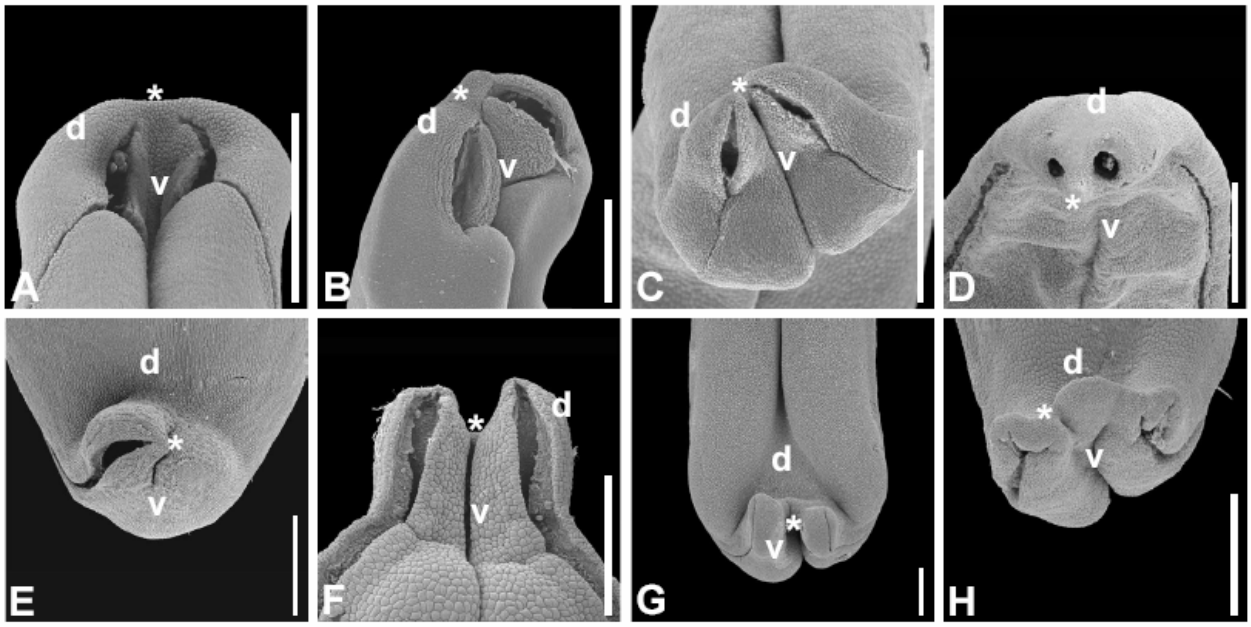
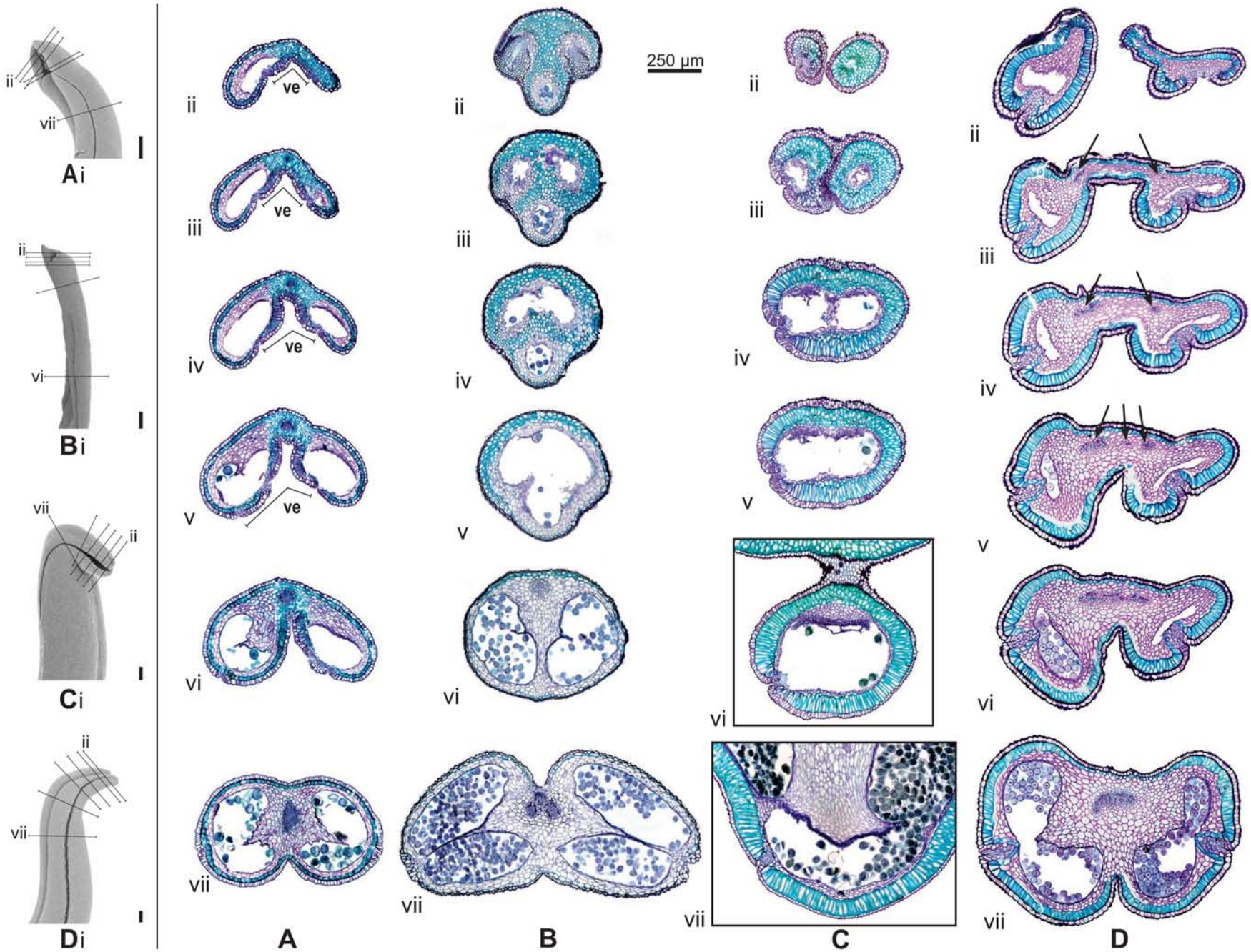


Fig. 1 Anther tips of lateral abaxial stamens in *Senna* species of clades I-III.

A, *S. polyantha* (I); B, *S. siamea* (I); C, *S. italica* (II); D, *S. nicaraguensis* (II); E, *S. paradictyon* (II); F, *S. unijuga* (III); G, *S. mollissima* (III); H, *S. wislizeni* (III). v, ventral anther side; d, dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 µm.

Fig. 2 Transverse section series of anther tips of abaxial stamens in *Senna* species of clades I-III.

A, *S. polyantha* (I); B, *S. paradictyon* (II); C, *S. nicaraguensis* (II); D, *S. wislizeni* (III). Ventral side (ve) of anther on left side in SEM micrographs, and on lower side or as indicated in transverse sections. Arrows indicate branching vascular bundles in *S. wislizeni*. Lines on SEM micrographs indicate the approximate level of the transverse sections. Scale bars = 250 µm. →


ii
vii
Ai
I
ii
vi
Bi
I
vii
ii
Ci
I
ii
vii
Di
I
ve
ve
ve
ve
250 µm
ii
iii
iv
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vi
vii
A
ii
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B
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vii
C
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vi
vii
D

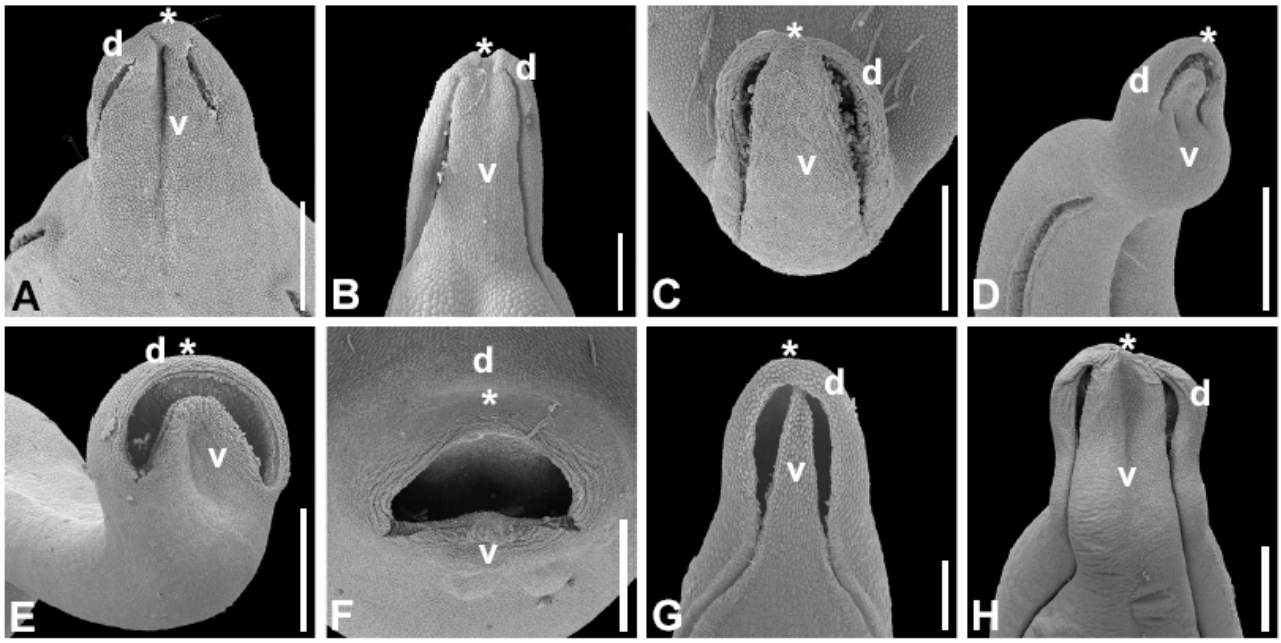
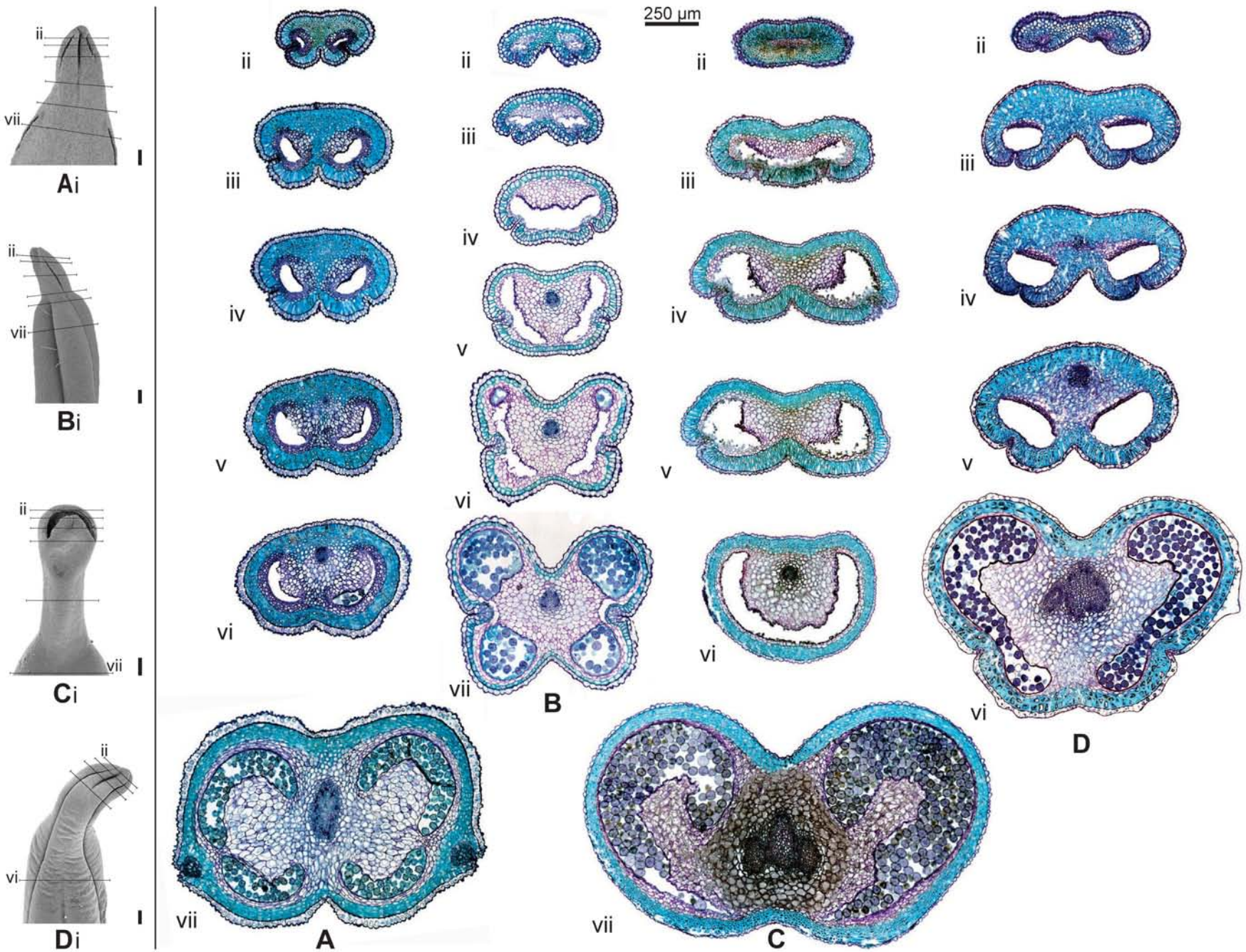


Fig. 3 Anther tips of abaxial stamens in *Senna* species of clades IV-V.

A, *S. skinneri* (IV); B, *S. aciphylla* (IVa); C, *S. macranthera* var. *nervosa* (IVb); D, *S. chloroclada* (IVb); E, *S. mucronifera* (IVb); F, *S. quinquangulata* (IVb); G, *S. uniflora* (V); H, *S. cana* var. *calva* (V). v, ventral anther side; d, dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 µm.

Fig. 4 Transverse section series of anther tips of abaxial stamens in *Senna* species of clades IV-V.

A, *S. skinneri* (IV); B, *S. aciphylla* (IVa); C, *S. mucronifera* (IVb); D, *S. cana* var. *calva* (V). Ventral side (ve) of anther on left side in SEM micrographs, and on lower side or as indicated in transverse sections. Lines on SEM micrographs indicate the approximate level of the transverse sections. Scale bars = 250. →


250 µm
ii
vii
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Ai
ii
vii
I
Bi
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vi
vii
I
Ci
ii
vi
I
Di
ii
iii
iv
v
vi
vii
A
ii
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v
vi
vii
B
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iii
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v
vi
vii
C
ii
iii
iv
v
vi
D

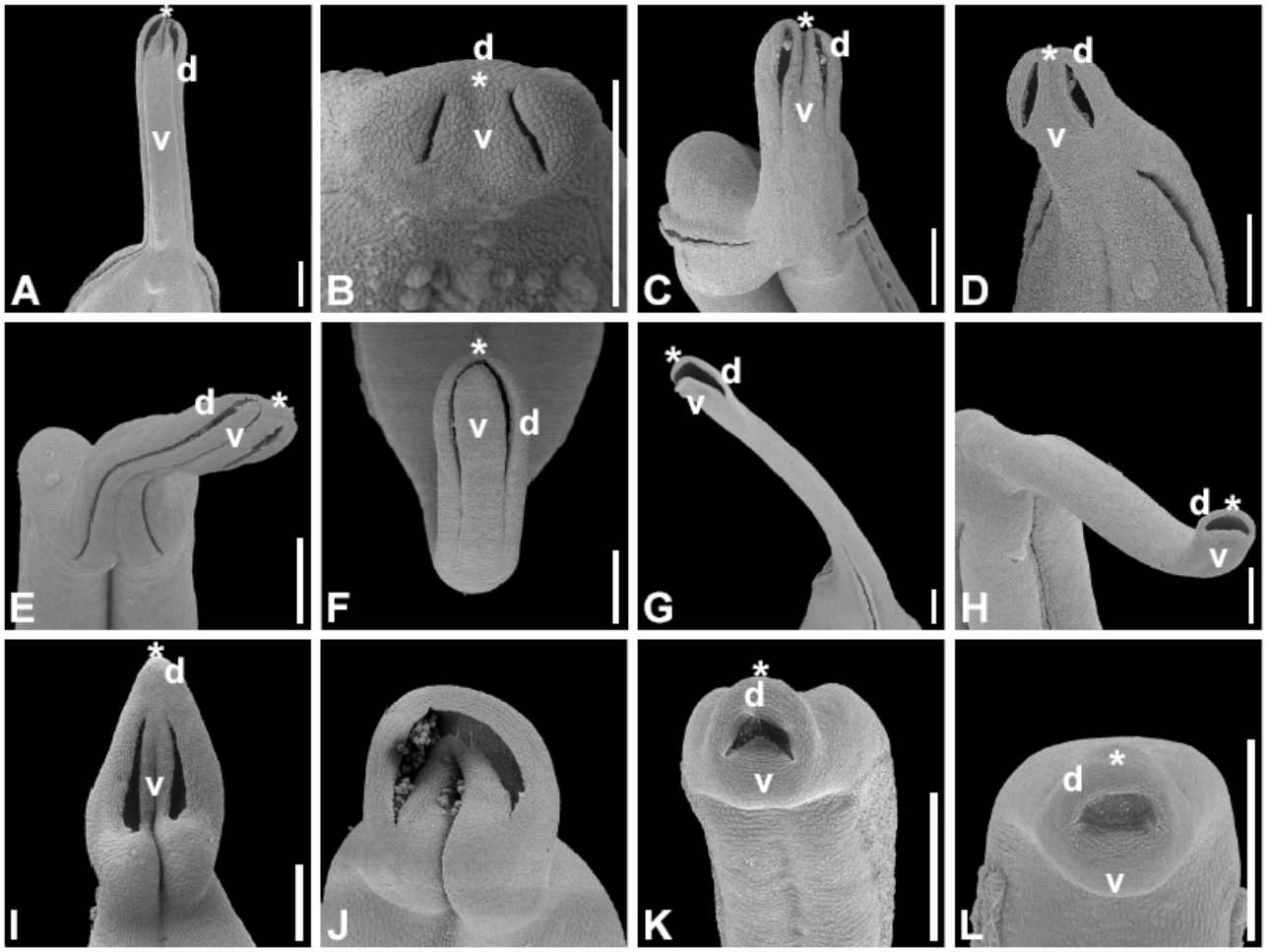


Fig. 5 Anther tips of lateral abaxial stamens in *Senna* species of clades VI-VII.

A, *S. acuruensis* var. *acuruensis* (VI); B, *S. chacoënsis* (VI); C, *S. multijuga* var. *multijuga* (VI); D, *S. rigida* (VI); E, *S. williamsii* (VI); F, *S. acuruensis* var. *catingae* (VI); G, *S. aversiflora* (VI); H, *S. tonduzii* (VI); I, *S. cernua* (VIIa); J, *S. pendula* (VIIa); K, *S. apiculata* (VIIb); L, *S. villosa* (VIIb). v, ventral anther side; d, dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 µm.

Fig. 6 Transverse section series of anther tips of abaxial stamens in *Senna* species of clades VI-VII.

A, *S. chacoënsis* (VI); B, *S. tonduzii* (VI); C, *S. cernua* (VIIa); D, *S. villosa* (VIIb). Ventral side (ve) of anther on left side in SEM micrographs, and on lower side or as indicated in transverse sections. Lines on SEM micrographs indicate the approximate level of the transverse sections. Scale bars = 250 µm. →

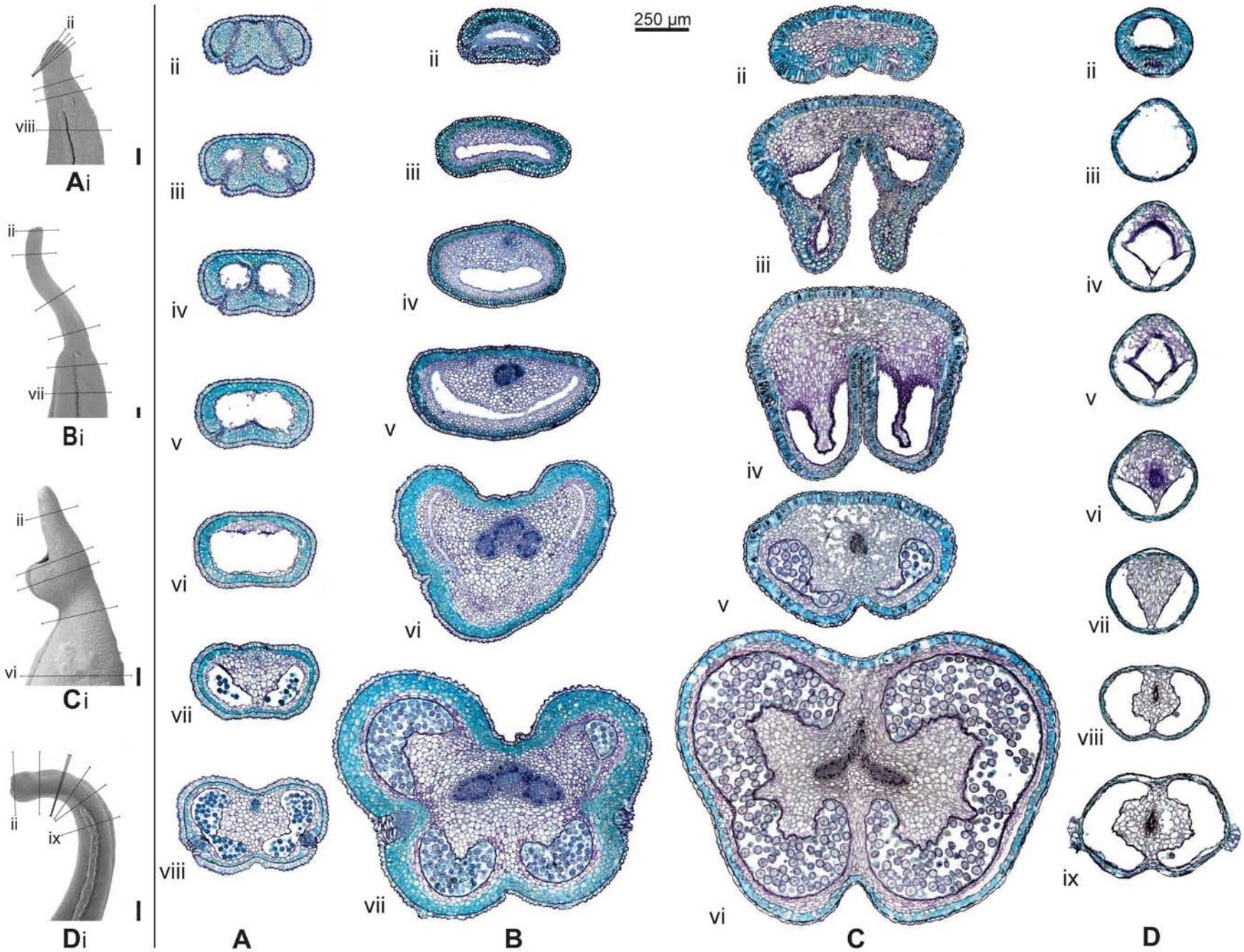
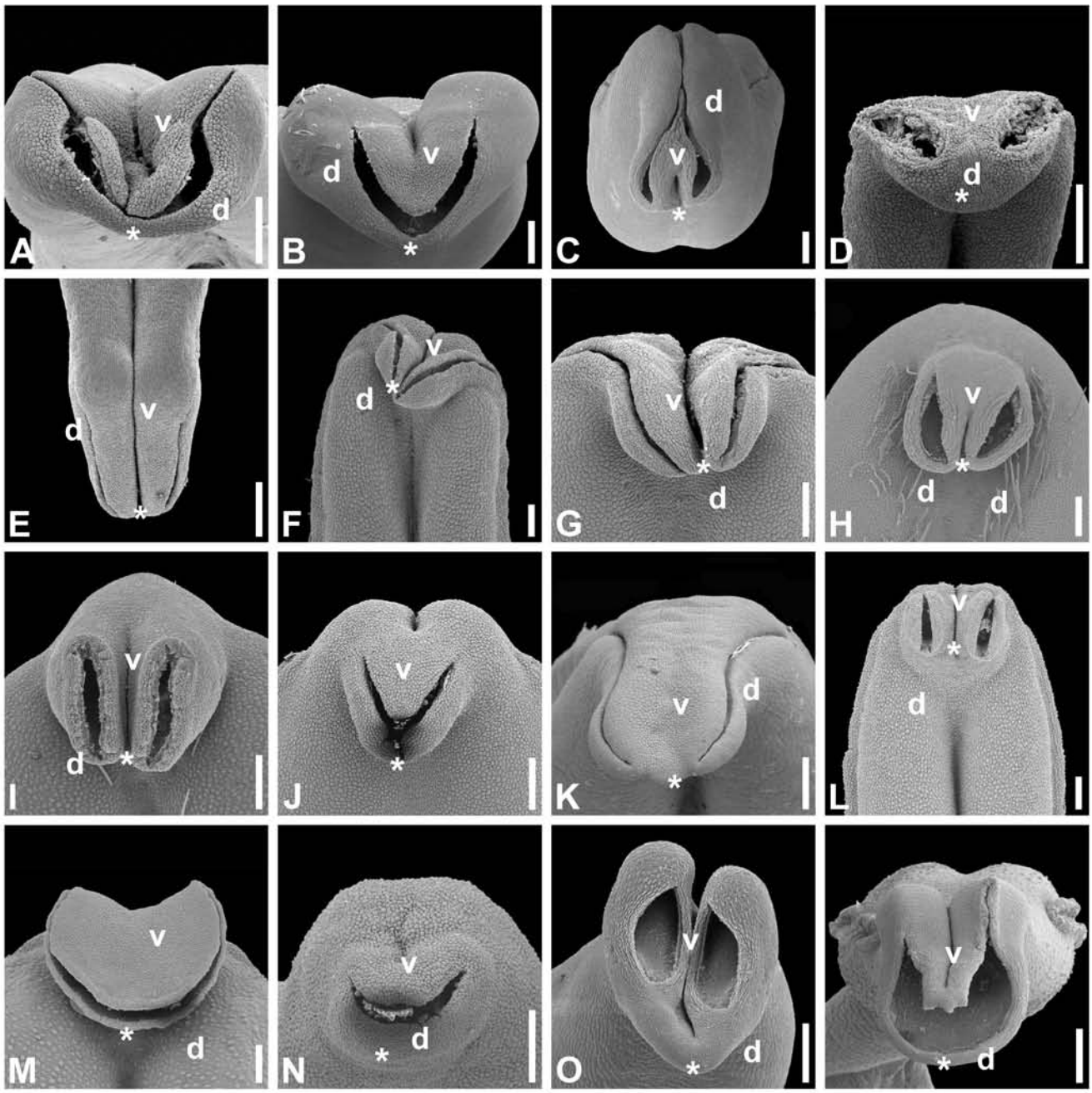

250 µm
Ai
Bi
Ci
Di
A
B
C
D

Fig. 7 Anther tips of middle stamens in *Senna* species of clades I-VII.

A, *S. polyantha* (I) (middle left stamen of inner whorl, iml); B, *S. siamea* (I) (iml); C, *S. nicaraguensis* (II) (middle right stamen of outer whorl, omr); D, *S. paradictyon* (II) (iml); E, *S. italica* (II) (middle right stamen of inner whorl, imr); F, *S. unijuga* (III) (middle left stamen of outer whorl, oml); G, *S. wislizeni* (III) (omr); H, *S. macranthera* var. *nervosa* (IVb) (iml); I, *S. mucronifera* (IVb) (iml); J, *S. skinneri* (IV) (imr); K, *S. cana* var. *calva* (V) (oml); L, *S. multijuga* var. *multijuga* (VI) (imr); M, *S. aversiflora* (VI) (omr); N, *S. tonduzii* (VI) (omr); O, *S. hirsuta* var. *leptocarpa* (VIIa) (iml); P, *S. pendula* (VIIa) (iml). v, ventral anther side; d, dorsal anther side. End of anther tip marked with asterisk. Scale bars = 250 µm.


A
v
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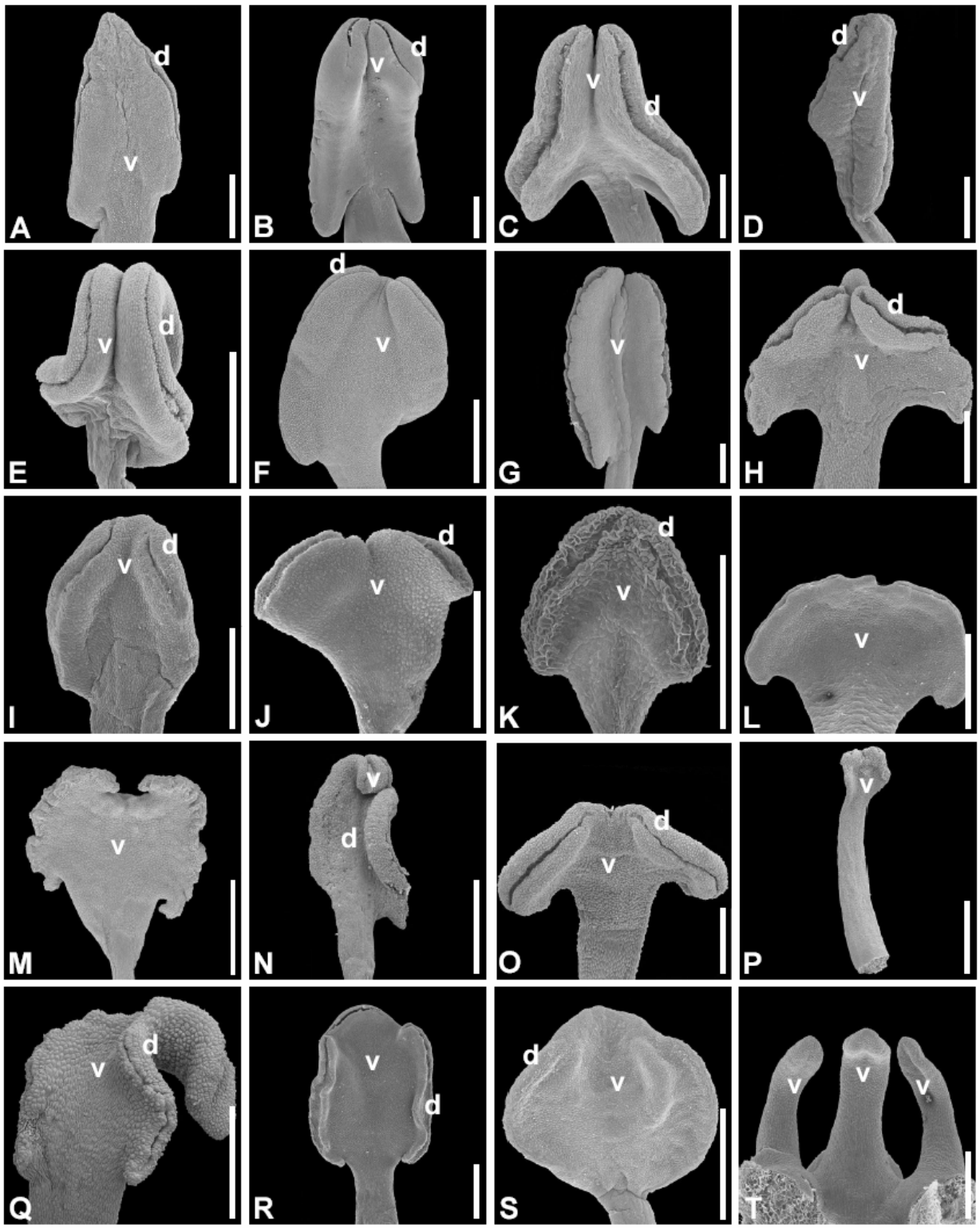


Fig. 8 Staminodes of *Senna*.

A, *S. polyantha* (I); B, *S. silvestris* var. *guaranitica* (I); C, *S. nicaraguensis* (II); D, *S. paradictyon* (II); E, *S. italica* (II); F, *S. atomaria* (III); G, *S. wislizeni* (III); H, *S. unijuga* (III); I, *S. skinneri* (IV); J, *S. artemisioides* (IVa); K, *S. mucronifera* (IVb); L, *S. hayesiana* (IVb); M, *S. macranthera* var. *nervosa* (IVb); N, *S. cana* var. *calva* (V); O, *S. tonduzii* (VI); P, *S. holwayana* (VI); Q, *S. pallida* (VI); R, *S. chacoënsis* (VI); S, *S. hirsuta* var. *leptocarpa* (VIIa); T, *S. villosa* (VIIb). v, ventral anther side; d, dorsal anther side. End of anther tip marked with asterisk. Scale bars A-J, L-T = 500 µm; scale bar K = 250 µm.

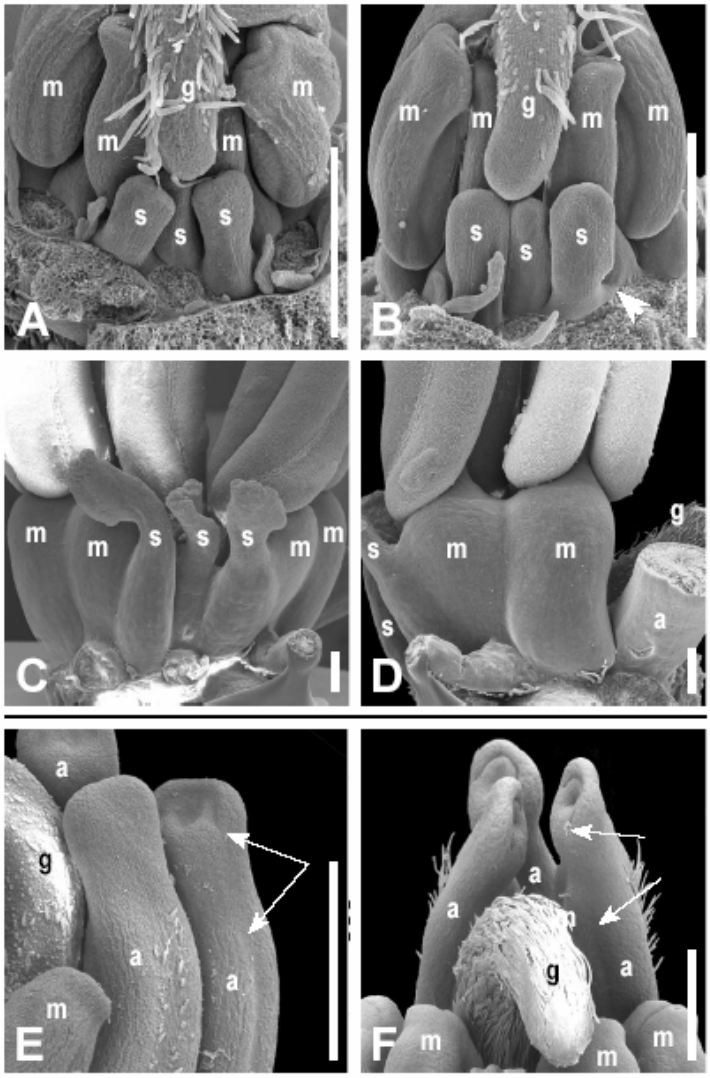


Fig. 9 Filament union and development of discontinuous anther lateral furrow in *Senna*.

A-D: Filament union in *S. tonduzii*. A, adaxial view of free filaments in young bud. B, adaxial view of later stage of development, with beginning filament union indicated by arrowhead. C, adaxial view of united filaments at anthesis; D, lateral view of united vs. free filaments at anthesis. E-F: Development of discontinuous lateral anther furrow in *S. mucronifera*. E, adaxial view of young abaxial stamens, continuous lateral furrow indicated by united arrows; F, lateral view of abaxial stamens at older stage, lateral furrow not connected to the pores indicated by separate arrows. Petals removed in all figures. a, abaxial stamen; g, gynoecium; m, middle stamen; s, staminode. Scale bars = 500 µm.

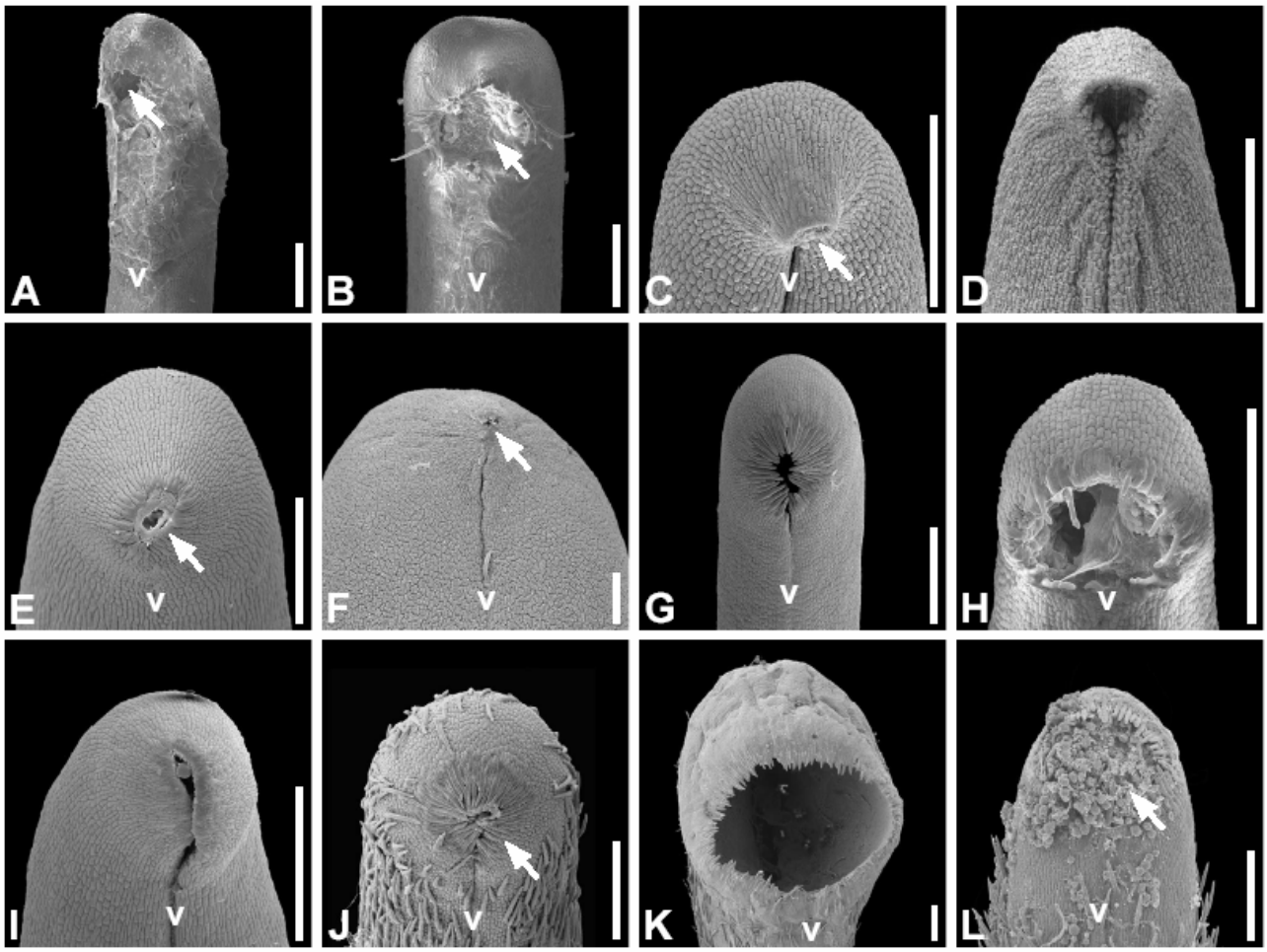


Fig. 10 Stigmas of *Senna* species of clades I-IV.

A, *S. polyantha* (I); B, *S. siamea* (I); C, *S. paradictyon* (II); D, *S. nicaraguensis* (II); E, *S. wislizeni* (III); F, *S. mollissima* (III); G, *S. skinneri* (IV); H, *S. artemisioides* (IVa); I, *S. aciphylla* (IVa); J, *S. mucronifera* (IVa); K, *S. quinquangulata* (IVb); L, *S. macranthera* var. *nervosa* (IVb). v, ventral anther side; d, dorsal anther side. Stigmatic orifice or surface indicated by arrow. Scale bars = 250 µm.

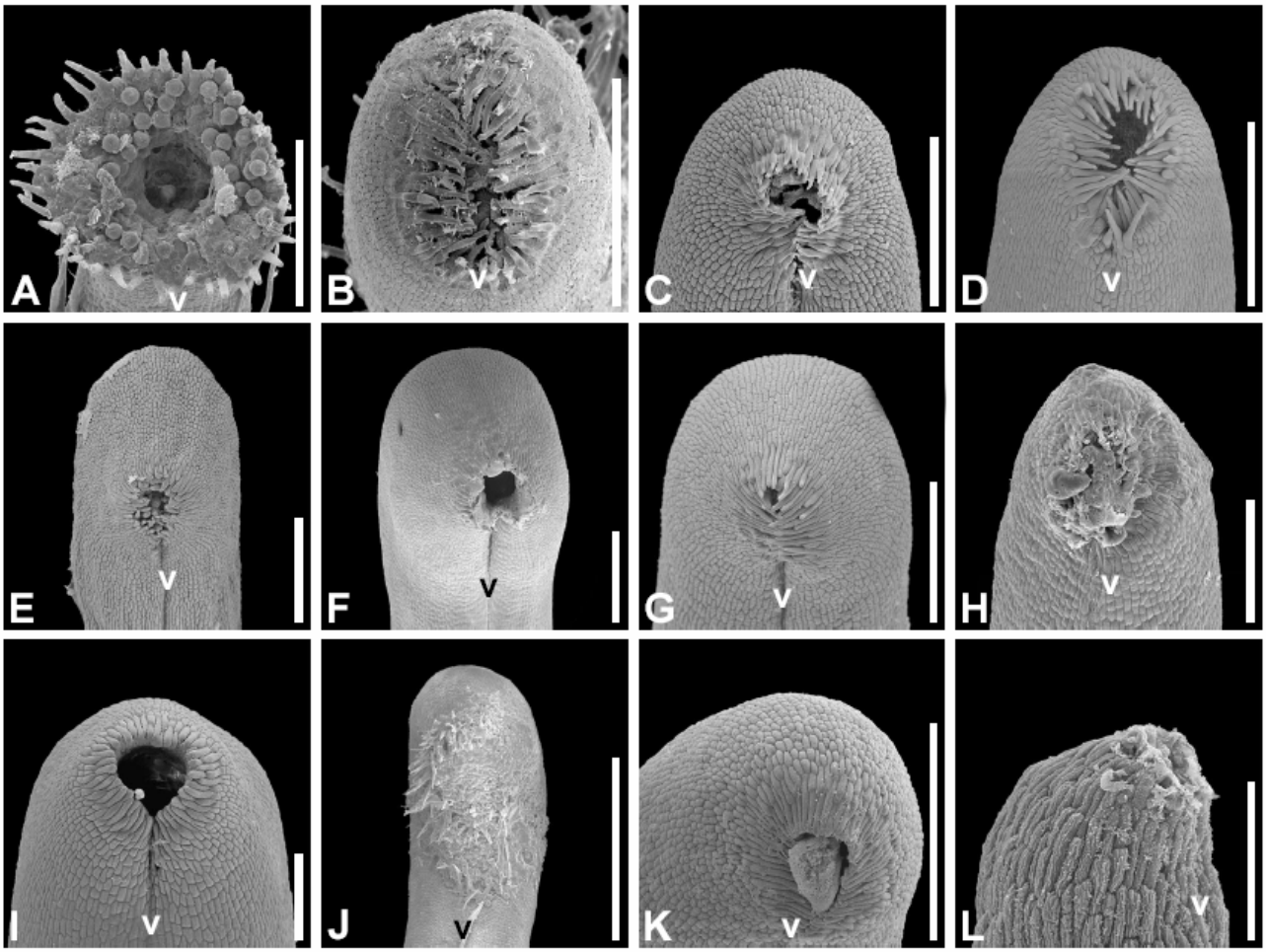
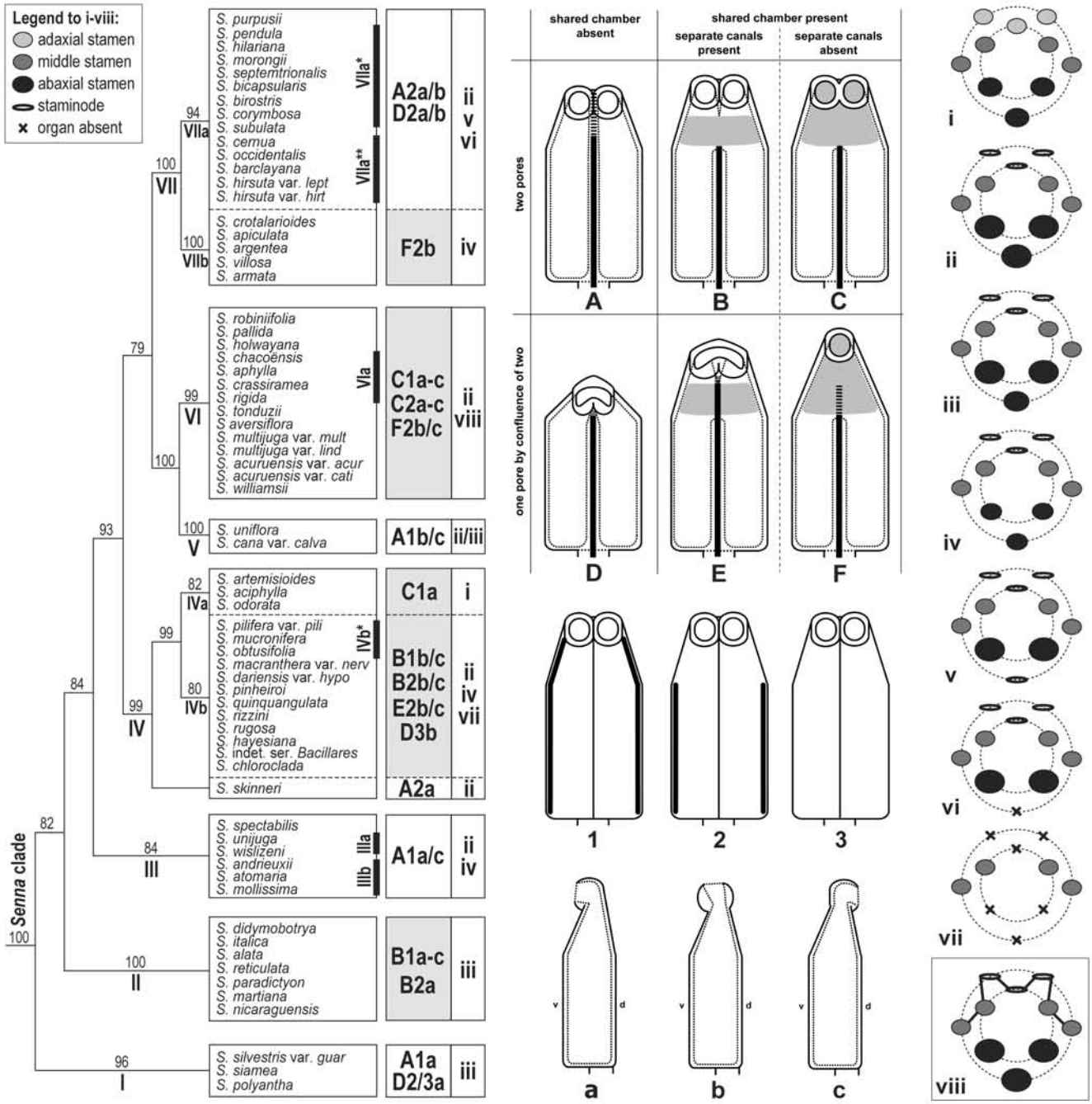


Fig. 11 Stigmas of *Senna* species of clades V-VII.

A, *S. uniflora* (V); B, *S. cana* var. *calva* (V); C, *S. williamsii* (VI); D, *S. pallida* (VI); E, *S. multijuga* var. *multijuga* (VI); F, *S. acuruensis* var. *acuruensis* (VI); G, *S. acuruensis* var. *catingae* (VI); H, *S. rigida* (VI); I, *S. aversiflora* (VI); J, *S. hirsuta* var. *leptocarpa* (VIIa); K, *S. pendula* (VIIa); L, *S. villosa* (VIIb). v, ventral anther side; d, dorsal anther side. Stigmatic orifice or surface indicated by arrow. Scale bars = 250 µm.

Fig. 12 Diagrams of different androecium patterns in *Senna* and their distribution on the molecular phylogenetic tree (simplified from Marazzi et al., 2006, fig. 2).

Phylogenetic tree: Bootstrap values above and clade numbers below the branches. Major clades represented by boxes, subclades separated by dotted lines, other major subclades supported in this study indicated by a longitudinal bold line. Relationships within clades or subclades not shown. Only species studied in this paper are listed (species in the same order as in Marazzi et al., 2006, fig. 2). In the boxes on the right of the tree, patterns shown in the diagrams are listed for clades and subclades. Grey boxes: patterns of anther dehiscence with shared chamber, open boxes: patterns lacking shared chamber. A-F: patterns of anther dehiscence (area of shared chamber in grey; vascular bundle in black). 1-3: extension of the lateral furrow (bold line). a-c: orientation of the anther tip and pointing direction of the dehiscence pore(s) or slits. i-vii: patterns of heteranthery; viii: stamen union in *S. tonduzii*.


Legend to i-viii:
adaxial stamen
middle stamen
abaxial stamen
staminode
organ absent
Senna clade
S. purpusii
S. pendula
S. hilariana
S. morongii
S. septemtrionalis
S. bicapsularis
S. birostris
S. corymbosa
S. subulata
S. cernua
S. occidentalis
S. barclayana
S. hirsuta var. lept
S. hirsuta var. hirt
VIIa*
VIIa**
VIIa
VIIb
VII
S. crotalarioides
S. apiculata
S. argentea
S. villosa
S. armata
S. robiniifolia
S. pallida
S. holwayana
S. chacoënsis
S. aphylla
S. crassiramea
S. rigida
S. tonduzii
S. aversiflora
S. multijuga var. mult
S. multijuga var. lind
S. acuruensis var. acur
S. acuruensis var. cati
S. williamsii
VIa
VI
S. uniflora
S. cana var. calva
V
S. artemisioides
S. aciphylla
S. odorata
IVa
S. pilifera var. pili
S. mucronifera
S. obtusifolia
S. macranthera var. nerv
S. dariensis var. hypo
S. pinheiroi
S. quinquangulata
S. rizzini
S. rugosa
S. hayesiana
S. indet. ser. Bacillares
S. chloroclada
IVb*
IVb
IV
S. skinneri
S. spectabilis
S. unijuga
S. wislizeni
S. andrieuxii
S. atomaria
S. mollissima
IIIa
IIIb
III
S. didymobotrya
S. italica
S. alata
S. reticulata
S. paradictyon
S. martiana
S. nicaraguensis
II
S. silvestris var. guar
S. siamea
S. polyantha
I
A2a/b
D2a/b
ii
v
vi
F2b
iv
C1a-c
C2a-c
F2b/c
ii
viii
A1b/c
ii/iii
C1a
i
B1b/c
B2b/c
E2b/c
D3b
ii
iv
vii
A2a
ii
A1a/c
ii
iv
B1a-c
B2a
iii
A1a
D2/3a
iii
shared chamber absent
shared chamber present
separate canals present
separate canals absent
two pores
one pore by confluence of two
A
B
C
D
E
F
1
2
3
a
b
c
v
d
i
ii
iii
iv
v
vi
vii
viii

PART 3

FLORAL ASYMMETRY: DIVERSITY, DEVELOPMENT AND EVOLUTION

Mirror flowers of *Senna aversiflora*.

DEVELOPMENT AND EVOLUTION OF FLORAL ASYMMETRY PATTERNS IN *SENNA* (LEGUMINOSAE, CASSIINAE)

Submitted in May 2007 to American Journal of Botany.

ABSTRACT

The buzz-pollinated genus *Senna* (Leguminosae) includes both species with monosymmetric and species with diverse asymmetric, enantiomorphic (enantiostylous) flowers, with left and right morphs on the same plant, and is thus ideal to study floral asymmetry. To assess homology of the patterns of floral asymmetry, we studied flower structure of 39 species from all clades of *Senna*, and development of four enantiomorphic species from different clades. The asymmetry morph of a flower is correlated with the direction of spiral calyx aestivation (clockwise: right morph; counter-clockwise: left morph). We recognized five major patterns of floral asymmetry, resulting from different combinations of at least six structural elements: (1) deflection of the carpel; (2) deflection of the median abaxial stamens; (3) deflection of one lateral abaxial stamen or, rarely, (4) modification in size of this stamen; and modification in shape and size of (5) one or (6) both lower petals. Petals have diverse venation: most *Senna* species have three main petal veins, a few species of the basal lineages of Senna, and also *Cassia* (sister to *Senna*), have only one main vein. Expression of floral asymmetry during development differs among floral whorls. Prominent corolla asymmetry begins in early bud (unequal development of lower petals). Androecium asymmetry begins in mid-stage bud (unequal development of thecae in median abaxial stamen; twisting of androecium) or at anthesis (stamen deflection). Gynoecium asymmetry begins in early bud (primordium appearing off the median plane; ventral slit laterally oriented) or mid-stage to late bud (carpel deflection). In both monosymmetric and highly asymmetric corollas of enantiostylous flowers, pronouncedly concave and robust petals likely function to ricochet pollen flow and to reduce loss of pollen during buzz pollination. Preliminary ancestral character state reconstructions moderately support the hypothesis that the carpel was not deflected in ancestral *Senna* flowers, but strongly support that asymmetric androecium and corolla evolved from enantiostylous flowers.

KEY WORDS

Buzz pollination, enantiomorphy, enantiostyly, floral development, floral symmetry, functional morphology, homology, petal venation,

INTRODUCTION

Asymmetric flowers are rare in angiosperms, and are known to occur mostly within large families or orders with predominantly monosymmetric (zygomorphic) flowers (e.g., Leguminosae, Lamiales, Orchidaceae, Zingiberales) and only exceptionally in basal angiosperms (e.g. Winteraceae; Endress 1999). Enantiomorphy is a special kind of floral asymmetry, in which flowers have two mirror-image morphs. In many enantiomorphic flowers, the style is deflected to the left or to the right of the median plane, a condition known as enantiostyly, which occurs in at least ten angiosperm families of both monocots and eudicots (Jesson 2002), and seems to be derived from monosymmetry multiple times (Jesson and Barrett 2003). Left- vs. right-styled flowers may occur on different individuals (i.e. dimorphic enantiostyly), or on the same plant (i.e., monomorphic enantiostyly; see Jesson and Barrett 2003, for an overview). The development of enantiostylous flowers has been explored in few taxa (Jesson et al. 2003b; Tucker 1996; 1999), while other studies focused on the genetics of enantiostyly (Jesson and Barrett 2002a and b), or its role in pollination biology (e.g., Delgado Salinas and Souza Sánchez 1977; Dulberger 1981; Gottsberger and Gottsberger-Silberbauer 1988). The large genus *Senna* (Cassiinae, Leguminosae)(ca. 350 species) is exceptional for displaying both species with monosymmetric flowers and species with enantiomorphic flowers (monomorphic), in which gynoecium, androecium, and corolla contribute to the floral asymmetry (Marazzi et al. 2006, submitted). *Senna* thus represents an ideal example to study floral asymmetry evolution.

Floral whorls in *Senna* affect floral structure and symmetry in various ways (Marazzi et al. 2006, submitted). The gynoecium is formed by a single carpel, as typical for legumes, and is usually long, arcuate and point-tipped with a chambered (enclosed receptive surface) or a craterlike stigma (receptive surface not enclosed; Owens and Lewis 1989; Dulberger et al. 1994; Endress 1994; Marazzi et al., submitted). Unlike most enantiostylous taxa, in *Senna*, not only the style, but the entire carpel is deflected to the side. The androecium consists of two five-merous whorls and is highly diverse, and has fascinated researchers since a long time (e.g. Müller 1883; Venkatesch 1956; Lasseigne 1979; Tucker 1991; Marazzi et al., submitted). Of the usually seven fertile stamens, only the three abaxial ones appear to be involved in floral asymmetry. The corolla is usually yellow and more or less differentiated into three upper (i.e. standard petal and wing petals) and two lower petals (i.e. keel petals). In several enantiostylous flowers, the upper petals are more or less reduced, and the lower ones concave and/or modified in shape and size (Irwin and Barneby 1982; Marazzi et al. 2006).

Expression of highly asymmetric species of *Senna* has not been investigated. Floral development has been studied in detail only in one species, *S. didymobotrya* (Tucker 1996), which has enantiostylous flowers with monosymmetric androecium and corolla. This species was part of a comparative study of *Senna*, *Cassia* s.str. and *Chamaecrista* (Tucker 1996), the three genera of subtribe Cassiinae (Irwin and Barneby 1981, 1982). Flowers of *Cassia* s.str. are monosymmetric, whereas all *Chamaecrista* species have asymmetric, enantiostylous flowers (Irwin and Barneby 1982, Tucker 1996). Cassiinae have superficially similar flowers at anthesis due to convergence to the same pollination syndrome, but differ in early floral development (Tucker 1996, 1997). Dulberger (1981) observed that in *S. didymobotrya* deflection of the carpel occurs 6-12 h before anthesis. In other enantiostylous species style deflection occurs either in bud or at the beginning of anthesis (Jesson et al. 2003b).

Enantiostyly is related to the pollination biology of buzz flowers, which are visited by pollen-collecting bees that vibrate the anthers to extract and collect the pollen for larval provision (i.e. buzz pollination; e.g. Buchmann 1974, 1983). Although enantiostyly was commonly interpreted as promoting cross-pollination, its functional significance has long been debated (e.g., Todd 1882, Dulberger 1981, Fenster 1995, Jesson & Barrett 2002a). Enantiostyly is associated with other floral features that likely have evolved in relation to buzz pollination, including poricidal anthers (i.e., dehiscence restricted to apical pores) and heteranthery (i.e., different kinds

of stamens in a flower), which are also found in *Senna* (e.g., Buchmann 1974; Delgado Salinas and Souza Sánchez 1977; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988; Owens and Lewis 1989; Dulberger et al. 1994). The role of these features and of enantiostyly in the pollination biology of *Senna* has been investigated only in a few species (Buchmann 1974; Delgado Salinas and Souza Sanchéz 1977; Fontanelle 1979; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003; Laporta 2003; Westerkamp 2004). Implications for pollination biology of specialized anther dehiscence patterns are discussed by Marazzi et al. (submitted).

In *Senna*, various kinds of floral asymmetry appear to occur and characterize clades II-VI. The existence of different kinds of asymmetric flowers and the independent switches to these kinds inferred from the molecular phylogeny suggest that such variants may have originated through different evolutionary and developmental pathways, therefore they are not strictly homologous (Marazzi et al. 2006). Floral symmetry appears rather as a syndrome of characters resulting from different combinations of features and may not be treated as a single character (Herendeen et al., 2003). Structural studies are necessary to dissect the floral symmetry syndrome in *Senna* into its component parts and differentiate patterns of homology in view of reconstructing the evolution of floral asymmetry in a phylogenetic framework. In the present study, we investigate the diversity in petals and patterns of floral symmetries observed in the genus, and in particular, the patterns in corolla morphology and development of floral asymmetry. We will address the following specific questions: (1) What patterns of floral asymmetry can be identified in *Senna*? (2) What patterns can be recognized in the diversity of petal form? (3) Do the investigated features provide any synapomorphies congruent with the new infrageneric relationships supported by the molecular phylogeny of *Senna* (Marazzi et al. 2006)? (4) How do species with different patterns of floral asymmetry differ in development? (5) What are the implications of our results to improve our understanding of pollination biology and floral symmetry evolution in *Senna*?

MATERIAL AND METHODS

Our taxonomic sampling includes 39 *Senna* species (one individual per species) and two *Cassia* species, representing the sister genus of *Senna* (Marazzi et al. 2006). Species of *Senna* represent the clades and subclades of the molecular phylogeny of the genus by Marazzi et al. (2006) based on 83 species, and the diversity of morphological patterns observed (see also Marazzi et al., submitted). Twenty-three species of *Senna* were selected for detailed investigations on petal shape and venation in anthetic flowers (seventeen of which, together with *Cassia javanica*, are shown in fig. 1), and four species with asymmetric flowers, *S. aciphylla* (clade IVa), *S. mucronifera* (clade IVb), *S. tonduzii* (clade VI), and *S. wislizeni* (clade III) were selected for developmental studies with Scanning Electron Microscopy (SEM), while the remaining species were investigated with stereomicroscopy (SM). In addition, floral buds at mid-stage of *S. aciphylla*, *S. tonduzii*, and *S. wislizeni* were selected for microtome sectioning. Color photographs were made in the field by the first author, except for three species, *S.* cf. *velutina* (Vogel) H.S. Irwin & Barneby and *S. subulata* (Griseb.) H.S. Irwin & Barneby, photographed by M. Belgrano (Instituto de Botánica Darwinion, Argentina), and *S. martiana* (Benth.) H.S. Irwin & Barneby, photographed by L. Paganucci de Queiroz (Universidade Estadual de Feira de Santana, Brazil). Information on the floral morphology of these three species is based on their color photographs. A list of the specimens studied and voucher information are given in the Appendix.

Flowers at anthesis and buds of different stages were fixed and stored in 70% ethanol. To investigate petal shape and venation, each petal was flattened between two glass slides with the ventral side downwards. The slides were then immersed into 70% ethanol and photographed with a Axiocam HRc digital camera (Carl Zeiss AG, Oberkochen, Germany) mounted on the Stemi SV11 stereomicroscope (Carl Zeiss AG). Standard specimen preparation procedures were used for osmium tetroxide impregnated samples for SEM studies. Specimens for serial microtome

sectioning were embedded in Kulzer's Technovit 2-hydroethyl methacrylate (Igersheim 1993) and sectioned with a Microm HM 335 rotary microtome (Microm International GmbH, Walldorf, Deutschland) and conventional microtome knife (grade D); transverse section series were cut at 7 μm, stained with ruthenium red and toluidine blue (Weber and Igersheim 1994), and mounted in Histomount on glass slides. Fixed floral material and slides are deposited at the Institute of Systematic Botany of the University of Zurich (Z), Switzerland.

RESULTS

Diversity of floral symmetry and petals

Results of our investigations on the floral whorls in *Senna* and *Cassia* are summarized in table 1, and a general floral diagram of *Senna* is given in fig. 4. In *Cassia*, the corolla is monosymmetric, and petals approximately have all the same size and form (fig. 1A), but the lower petals are slightly concave. Petals have a single main vein, which extends up to the petal tip. It must be noted that the sepals are arranged either in a clockwise or counter-clockwise spiral in all *Senna* species, which means, that monosymmetric flowers of *Senna* also have an enantiomorphic calyx. In the following paragraphs we describe floral symmetry and petal diversity in the major clades of the molecular phylogeny of *Senna* (Marazzi et al. 2006).

CLADE I - Flowers are monosymmetric. The gynoecium may be slightly deflected to the side in some flowers, but because the deflection is inconsistent in these species, their flowers are not considered truly enantiostylous. Upper and lower petals have similar forms, but the lower petals are slightly larger than the upper ones (figs. 1B, C). All petals are only slightly concave (figs. 2A, B). Petals apparently have a single main vein, which does not extend up to the petal tip.

CLADE II - Flowers are asymmetric, with the gynoecium deflected to the side in all species investigated, while the androecium and corolla are monosymmetric. Upper and lower petals have more or less the same shape, but the upper petals are wider (*S. paradictyon*, fig. 1D) or smaller (*S. didymobotrya*, fig. 1E) than the lower ones. All petals are concave (*S. alata*), or the standard petal is more pronouncedly concave than the other petals (*S. didymobotrya*, *S. nicaraguensis*, fig. 2C, *S. martiana*, fig. 2D), or only the standard petal is concave and the lower ones are folded upwards (i.e. U-shaped; *S. paradictyon*, fig. 2E). Interestingly, the petals are short-stalked (e.g., *S. alata*, *S. didymobotrya*, fig. 1E), or the standard petal only appears stalkless (*S. paradictyon*, fig. 1D). Petals have a single main vein (*S. paradictyon*, fig. 1D), or three main veins (*S. didymobotrya*, fig. 1E), which in both cases do not extend up to the petal tip.

Clade III - Flowers are asymmetric, with the gynoecium deflected to the side in all species investigated. The androecium is nearly monosymmetric (species of subclade IIIb, *S. spectabilis*) or the median and one lateral abaxial stamen are deflected to the opposite side of the gynoecium (species of subclade IIIa). The corolla is asymmetric, whereby the upper petals are not reduced (*S. spectabilis*, *S. unijuga*, fig. 1F, *S. wislizeni*, fig 1G), or moderately reduced (*S. atomaria*, fig. 2 F, *S. mollissima*, fig. 1H), and one lower petal (*S. atomaria*, *S. mollissima*, fig. 1H, *S. spectabilis*) or both lower petals (*S. unijuga*, figs. 1F, 2G, *S. wislizeni*, figs. 1G, 2H) are modified in shape and size, i.e. the blade is highly asymmetric, concave and 'foot-shaped' (figs. 1F-H). The standard petal appears stalkless in *S. mollissima* (fig. 1H). Upper petals have three main veins (*S. unijuga*, fig. 1F, in *S. wislizeni*, fig. 1G, the median vein is more conspicuous than the lateral ones) and the lower petals have apparently only two main veins (*S. unijuga*, fig. 1F, *S. wislizeni*, fig. 1G), or all petals have a single main vein, except the standard petal, in which two main veins run close to one another, forming a double strand (*S. mollissima*, fig. 1H). In strongly modified petals, venation is particularly robust, and the basal part of the main veins is united (figs. 1F, G), or many robust secondary veins extend from the basal part of the single main vein (fig. 1H).

CLADE IV - Flowers are asymmetric, with the gynoecium deflected to the side in all species investigated. This clade includes *S. skinneri* plus a clade of two subclades IVa and IVb (Marazzi et al. 2006). The androecium of subclade IVa is asymmetric: all the stamens are fertile

and are arranged slightly irregularly (*S. aciphylla*, fig. 2I, *S. artemisioides*, fig. 2J), and one lateral abaxial stamen may be larger than the others (*S. artemisioides*, fig. 2I). *Senna skinneri* and species of subclade IVb have seven fertile stamens, except *S. hayesiana* with only the four middle stamens fertile. The androecium is asymmetric, with only the median abaxial stamens deflected to the opposite side of the deflected gynoecium (*S. skinneri*, fig. 2K, species of subclade IVb, figs. 2L-N) or it is nearly monosymmetric (*S. hayesiana*, fig. 2O, *S. quinquangulata*, fig. 2P). The corolla is asymmetric in most species (subclade IVa, figs. 2I-J, species of subclade IVB and *S. skinneri*, figs. 2K-N), or, rarely, it is nearly monosymmetric (*S. hayesiana*, fig. 2O, *S. quinquangulata*, fig. 2P). In asymmetric corollas, the upper petals are not reduced, and one or both lower petals are concave, but not modified in shape and size (species of subclade IVa, figs. 1I, 2I-J, species of subclade IVb, figs. 2K, N), or one or both are concave and foot-shaped (species of subclade IVb, figs. 1J, 2L-M). The standard petal may be emarginate or bilobed (species of subclade IVb*, fig. 1J). Petals have three main veins (figs. 1I, J). In strongly modified petals, venation is particularly robust, and the basal part of the main veins is united (fig. 1J).

CLADE V - Flowers are asymmetric, with the gynoecium deflected to the side in all species investigated. The androecium is nearly monosymmetric in species with the median abaxial stamen smaller than the lateral abaxial ones (*S.* cf. *velutina*, fig. 3A), or it is asymmetric in species with three similar abaxial stamens, and the median abaxial stamen deflected to the opposite side of the gynoecium (*S. cana* var. *calva*, *S. uniflora*, fig. 3B). The corolla is nearly monosymmetric (*S.* cf. *velutina*, fig. 3A, *S. uniflora*, figs. 1K, 3B), or slightly asymmetric, with one lower petal concave and slightly modified in shape and size (*S. cana* var. *calva*, fig. 1L). The standard petal may be emarginate (fig. 1L). Petals have three main veins (fig. 1L), or, in *S. uniflora*, they apparently have only a single main vein (fig. 1K). In the lower petal that is modified, venation is slightly more robust than in the other petals, but the basal part of the main veins appears to be free (fig. 1L).

CLADE VI - Flowers are asymmetric. The androecium is asymmetric in all species investigated: all three abaxial stamens are deflected to the opposite site of the gynoecium (*S. aversiflora*, fig. 3D, *S. holwayana*, fig. 3E, *S. tonduzii*, fig. 3F), or only the median abaxial stamen is deflected, and the lateral abaxial stamen opposite to the gynoecium is longer (*S. acuruensis* var. *acuruensis*, fig. 3G, *S. multijuga* var. *multijuga*, fig. 3H), or the lateral abaxial stamens opposite to the gynoecium is conspicuously longer than the other two abaxial stamens which are not deflected (species of subclade VIa, figs. 3J-L), or rarely, the two lateral abaxial stamens are deflected to one side and the median abaxial stamen to the other side (*S. pallida*, fig. 3I). The corolla is asymmetric. Upper petals are highly reduced (*S. acuruensis*, fig. 1M, *S. tonduzii* fig. 1N), or moderately reduced (*S. pallida*, figs. 1O, 3I), or almost not reduced (*S. aversiflora*, fig. 3D, *S. holwayana*, *S. multijuga* var. *multijuga*, fig. 3H, *S. aphylla*, fig. 3J, *S. chacoënsis*, figs. 1P, 3K, *S. rigida*, fig. 3L). In reduced upper petals, the standard petal is the most reduced, whereas the lateral ones are similar to one another (*S. acuruensis* var. *acuruensis*, fig. 1M), or the lateral petal opposite the gynoecium is smaller than the other lateral upper petal (*S. pallida*, figs. 1O, 3I, *S. tonduzii* fig. 1N). The lower petal opposite the gynoecium is concave and foot-shaped in all species investigated, whereas the other lower petal is concave and modified in size, but not foot-shaped, and only slightly asymmetric (*S. aversiflora*, fig. 3D, *S. holwayana*, fig. 3E, *S. acuruensis* var. *acuruensis*, figs. 1M, 3G, *S. multijuga* var. *multijuga*, fig. 3H, *S. pallida*, figs. 1O, 3I, *S. aphylla*, fig. 3J, *S. chacoënsis*, figs. 1P, 3K, *S. rigida*, fig. 3L), or it is strongly asymmetric (*S. tonduzii*, figs. 1N, 3F). Petals have three main veins (figs. 1M-P), except for highly reduced upper petals, which have only one main vein (figs. 1M, N).

CLADE VII - Flowers are monosymmetric in both subclades VIIa and VIIb. In some flowers of subclade VIIa, the gynoecium may be slightly deflected to the side, but because the deflection is inconsistent in these species, their flowers are not considered truly enantiostylous. Upper and lower petals have similar shapes (*S. apiculata*, *S. birostris*, *S. villosa*, figs. 1Q, 3P), or the lower petals are slightly longer and thinner than the upper ones and are concave (*S. hirsuta*,

figs. 1R, 3M, *S. septemtrionalis*, fig. 3N, *S. subulata*, fig. 3O). In *S. villosa*, the petals are short-stalked (fig. 1Q). The standard petal is emarginate (figs. 1Q, R, 3M-P). The blade of the upper lateral petals is monosymmetric (e.g., *S. hirsuta*, fig. 1R), or slightly asymmetric, forming two mirror-image petals (e.g., *S. pendula*). Petals have three main veins (figs. 1Q, R).

Floral development

We focus on the developmental stages at which the floral asymmetry becomes apparent especially in the corolla and androecium. We studied young stages of representatives of four clades that are characterized by asymmetric flowers, *S. wislizeni* (clade III; fig. 5), *S. aciphylla* (subclade IVa, fig. 6), *S. mucronifera* (subclade IVb, fig. 7), and *S. tonduzii* (clade VI, fig. 8) with the SEM and transverse sections of mid-stage floral bud of each representative species, except *S. mucronifera* (figs. 4A-C). A detailed ontogenetic study of *S. didymobotrya*, a member of clade II, was carried out by Tucker (1996), including comparison with a few other *Senna* species (*S. artemisioides*, clade IVa; *S. auriculata*, clade uncertain; *S. bicapsularis*, clade VIIa; *S.* x *floribunda*, clade uncertain; *S. obtusifolia*, clade IVb; *S. occidentalis*, clade VIIa; and *S. surattensis*, clade uncertain).

We found a strong correlation between the direction of spiral calyx aestivation and the direction of deflection of the carpel at anthesis in all species studied developmentally: a clockwise spiral (i.e. fifth sepal on the right of adaxial floral side) corresponds to a right floral morph, while a counter-clockwise spiral (i.e. fifth sepal on the left of adaxial floral side) corresponds to a left floral morph. Therefore, the calyx aestivation allows prediction of the floral morph in buds.

***Senna wislizeni* (clade III)** – In anthetic flowers of *S. wislizeni* the carpel is deflected to the side, the median and one lateral abaxial stamen are deflected to the opposite side of the carpel, and the corolla is asymmetric, with the upper petals not reduced and both lower petals concave and foot-shaped. Blade modification is more conspicuous in the lower petal opposite the deflected carpel (figs. 1G, 2H).

The first sepal to be initiated is in the median abaxial position (fig. 5A). Lower petals are initiated before upper petals. They develop unequally, and are early of different size, and, thus, the corolla asymmetric (fig. 5B). Organs of the outer androecial whorl are initiated when petals start to develop (fig. 5C), while organs of the inner androecial whorl are initiated after the organs of the outer whorl start to develop (compare figs. 5E, F). The carpel is initiated as a bulge in the center of the bud, during initiation of the organs of the outer androecial whorl (figs. 5C, E).

In early bud, lower petals are larger than upper petals, and the shape of one lower petal begins to differentiate into a foot-like shape (fig. 5D). Corolla aestivation is quincuncial (see fig. 4D), with the lateral upper petals covering the median upper petal (i.e., standard petal) and also the lower petals (fig. 5D). The androecium is still monosymmetric after all organs of the outer androecial whorl are initiated (fig. 5E). With subsequent anther development, the androecium becomes asymmetric, as the stamens and staminodes of the outer androecial whorl appear to be arranged in a contort pattern, i.e. with the anther tips of the middle and median abaxial stamens touching the side of the subsequent neighboring anther tip (fig. 5F). This contort pattern disappears with development of the abaxial stamens of the inner whorl (fig. 5G). Differentiation of the thecae in the median abaxial stamen is unequal, one theca becoming larger than the other one (figs. 5G-I). In mid-stage bud, stamens of the inner and outer whorls are of different size (figs. 5H, K). In late bud, all middle stamens are of similar size, while the median abaxial stamen is the largest of the abaxial stamens (figs. 5I, L). During anther differentiation, the carpel becomes arcuate and is still in the plane of floral monosymmetry up to mid-stage bud (figs. 5G, H, J, K), while it appears slightly deflected in late bud (figs. 5I, L).

***Senna aciphylla* (clade IVa)** – In anthetic flowers of *S. aciphylla*, the carpel is deflected to the side, all the stamens are fertile and are deflected in an irregular manner, the corolla is

asymmetric, with the upper petals not reduced, and one lower petal concave, but not modified in shape and size (figs. 1I, 2I).

Sepals are initiated spirally (fig. 6A, B). The lower petals and lateral upper petal are initiated before the two remaining upper petals (fig. 6C). Initiation of these upper petals nearly overlaps with initiation of the stamens of the outer androecial whorl, except one adaxial stamen, which will be initiated later in front of the fifth sepal, between the two upper petals that initiated at least (fig. 6C). Abaxial stamens of the inner androecial whorl are initiated before the middle and adaxial stamens (fig. 6D). The carpel is initiated after the stamens of the outer androecial whorl and abaxial stamens of the inner whorl, but before the middle and adaxial stamens of the inner whorl (fig. 6D).

In early bud, the lower petals are already larger than the upper petals, and, although the upper petals are of different size, the corolla appears nearly monosymmetric (fig. 6D). Subsequently, the upper petals become of similar size, and corolla aestivation becomes quincuncial (fig. 6E; see also figs. 4B, D). During anther development, one middle and the two adaxial stamens of the outer androecial whorl appear to be arranged in a contort pattern (figs. 6F, G, J). This contort pattern disappears with development of the abaxial stamens of the inner whorl in mid-stage bud (fig. 6H). At this stage, anther differentiation (and anther tip elongation) first begins in the stamens of the outer whorl and in the abaxial stamens of the inner whorl (figs. 6H, I, K). Stamens of the outer whorl are larger than those of the inner whorl also in late bud (figs. 6I, L). Although differentiation of the thecae in the median abaxial stamen is slightly unequal, and one theca becomes thus larger than the other one, the androecium appears to be still nearly monosymmetric, but becomes irregularly asymmetric in late bud (figs. 6H, I, K, L). The gynoecium appears asymmetric in early bud, since the ventral slit is slightly oriented laterally (figs. 6E, F). During anther differentiation, the carpel becomes arcuate and appears slightly deflected in mid-stage bud (figs. 6H, I).

***Senna mucronifera* (clade IVb)** – In anthetic flowers of *S. mucronifera*, the carpel is deflected to the side, the androecium is asymmetric, with the median abaxial stamen deflected to the opposite side of the deflected carpel, and the corolla is asymmetric, with the upper petals not reduced, and both lower petals concave, one of which foot-shaped and the other only slightly asymmetric (figs. 1J, 2M).

The first sepal to be initiated is median abaxial (fig. 7A). The lower petals are initiated slightly before upper petals and are of different size; the corolla is thus already asymmetric (fig. 7B). The organs of the outer androecial whorl are initiated when the petals begin to develop (fig. 7C), while te organs of the inner androecial whorl are initiated after the organs of the outer whorl begin to develop (compare figs. 7C, D). Carpel initiation occurs after initiation of the organs of the outer androecial whorl (not shown, but compare figs. 7C, D).

In early bud, the lower petals are larger than the upper petals, and one lower petal begins to attain a foot-shaped (figs. 7C, D). Aestivation of the corolla is cochlear ascending, with one lower petal covering both its neighboring upper and lower petals (fig. 7E). The androecium is nearly monosymmetric after the organs of both the outer and inner androecial whorls are initiated (fig. 7F). With subsequent anther development, the androecium becomes asymmetric, as differentiation of the thecae in the median abaxial stamen is unequal, one theca becoming larger than the other one (figs. 7F-I). Stamens are all of approximately similar size in early bud, only the median abaxial stamen is much larger (fig. 7F). In mid-stage bud, the three abaxial stamens become much larger than the four middle stamens and the three adaxial staminodes (figs. 7G, H, J, K). However, at this stage, the stamens of the inner whorl are still slightly smaller than those of the outer whorl, and become of similar size in late bud (figs. 7I, L). In late bud, the anther tips of the abaxial stamens begin to elongate. During anther differentiation in mid-stage bud, the carpel becomes arcuate and appears only slightly deflected (see adaxial view of style, figs. 7J, K), but it becomes more deflected in late bud (figs. 7I, L).

***Senna tonduzii* (clade VI)** – In anthetic flowers of *S. tonduzii*, the carpel is deflected to the side, the androecium is asymmetric, with all the abaxial stamens deflected to the opposite side of the carpel, and the corolla is asymmetric, with the upper petals highly reduced, and one lower petal concave and foot-shaped, and the other with a strongly asymmetric blade (figs. 1N, 3F). Stamen union occurs in *S. tonduzii*, but in no other *Senna* species studied. The filaments of the three adaxial staminodes and four middle stamens are united (Marazzi et al., submitted).

The first sepal to be initiated is median abaxial, the other follow in a spiral sequence (fig. 8A). The two lower and one of the upper petals appear to be initiated before the two upper petals adjacent the fifth sepal (fig. 8B). The lower petals are of slightly different size; the corolla is thus early asymmetric (fig. 8B). The organs of the outer androecial whorl are initiated before the last two upper petal initials begin to develop (fig. 8C), while the organs of the inner androecial whorl are initiated when organs of the outer whorl begin to develop (compare figs. 8B, E). The carpel is initiated after initiation of the organs of the inner androecial whorl, and, interestingly, it appears to be displaced from the median plane of floral symmetry (fig. 8E).

In early bud, the lower petals are conspicuously larger than the upper petals, and the shape of one lower petal begins to become foot- shaped, and the other lower petal becomes asymmetric (figs. 8C, D). Aestivation of the corolla seems not to be cochlear ascending, since the upper petals appear in a partial contort pattern (fig. 8H). The androecium is nearly monosymmetric after all organs of both androecial whorls have been initiated (figs. 8E, F). With subsequent anther development, the androecium becomes asymmetric, as differentiation of the thecae in the median abaxial stamen is unequal, one theca becoming larger than the other one (fig. 8G). The stamens are all of approximately similar size in early bud, except the adaxial staminodes, which remain much smaller (fig. 8G). In mid-stage bud, the three abaxial stamens become larger than the middle stamens (fig. 8I). However, at this stage, the stamens of the inner whorl, and especially the middle ones, are still smaller than those of the other whorl, and become of almost similar size in late bud (fig. 8K). During anther differentiation the androecium becomes twisted, and is thus asymmetric (compare figs. 8G, I, J). Elongation of the anther tips begins in late bud (fig. 8J-L). Filament union begins in at late mid-stage bud (figs. 8K-L). During anther differentiation in mid-stage bud, the carpel becomes arcuate and deflected (figs. 8I). The deflection becomes stronger in late bud (figs. 8J, K).

DISCUSSION

Patterns of floral (a)symmetry

About half of the approximately 350 species of *Senna* have asymmetric, enantiostylous flowers, with both left and right morphs on the same plant. A result of our study is that the morph of a flower is correlated with the direction of spiral calyx aestivation: a clockwise spiral corresponds to a right morph, and a counter-clockwise spiral to a left morph. The morph can thus be predicted in floral bud by observing its calyx aestivation. Whether such a correlation occurs in other monomorphic enantiostylous genera has, to our knowledge, not been investigated. An enantiomorphic calyx is, however, present in all *Senna* flowers, including the monosymmetric ones. Species with asymmetric flowers occur in clades II-VI, while those with monosymmetric flowers characterize clades I and VII (table 1). We recognized six major patterns of floral (a)symmetry, which we describe in the following from the simplest to the most complex (table 2).

Pattern 1 corresponds to monosymmetric flowers. The petals are usually flat (*Senna* clades I, VII, *Cassia*), or, rarely, the lower petals may be concave (species of subclade VIIa). Pattern 2, the simplest pattern of floral asymmetry, involves the lateral deflection of the gynoecium only, while the androecium and corolla are monosymmetric (clades II, few species of subclade IVb). In pattern 3, floral asymmetry involves the corolla, in addition to the lateral deflection of the gynoecium, while the androecium is nearly monosymmetric (subclade IIIa) or slightly irregular (species of clade IVa). This androecial irregularity does not pertain to lateral deflection of

stamens, nor to modification of stamen size, but probably to differences in size among stamens and space constraints during development. Corolla asymmetry is due to concavity of the lower petal opposite the deflected carpel (e.g., *S. aciphylla*, figs. 1I, 2I). In patterns 4 and 5, patterns of intermediate complexity, floral asymmetry involves the lateral deflection of the carpel, the deflection of the median abaxial stamen (pattern 4; *S. skinneri*, species of clades IVb, V, VI), or the modification in size of the lateral abaxial stamen opposite the deflected carpel (pattern 5; species of subclades IVa, VIa), and the modification in shape and/or size of petals. In these patterns the corolla displays an array of modifications of the lower petals, from one lower petal concave to both lower petals highly concave and modified in size and shape, i.e. foot-shaped (table 2). In pattern 6, the most complex pattern of asymmetry, deflection of the carpel, of both the median and one lateral abaxial stamens, and modification in size and shape of the lower petals contribute to the floral asymmetry (species of clades III, VI). The asymmetric corollas are diverse: the upper petals are not reduced and one lower petal (e.g., *S. aversiflora* of clade VI) or both lower petals (e.g., *S. wislizeni* of subclade IIIa) are foot-shaped, or the upper petals are reduced, one lower petal is foot-shaped and the other lower petal is either concave and monosymmetric or flat and asymmetric (e.g., *S. acuruensis* var. *acuruensis* and *S. tonduzii*, respectively, both of clade VI).

Diversity in the corolla is particularly interesting in *Senna*, as petals have undergone diverse morphological modifications, and different patterns of venation occur. The modifications include (table 2): (1) reduction of upper petals (clades IIIb, VII, excluding VIa), (2) emarginate or bilobed shape of standard petal (clades V, VII and species of IV), (3) concavity of the standard petal (clade II), (4) concavity of lower petals (species of clades III-VII), (5) enlargement and modification into asymmetric shape of lower petals (clades III, VI, and species of clade IVb). Our results do not support Tucker's (1997, p. 160) observation, that the enlarged petal in *Senna* is a lateral upper ("wing") petal. The latter two kinds of petal modification contribute to most of the diversity observed in corolla asymmetry. In addition, in the first kind of modification, the upper petals may be unequally reduced, i.e. the lateral upper petals are of slightly different size, but this unequal reduction only weakly affects floral asymmetry. Moreover, they are often partially hidden by the enlarged lower petals. Strong petal modification causing asymmetric corollas is found also in the lower petals of *Chamaecrista* (Tucker 1996, 1997).

Petal venation in *Senna* is interestingly diverse (fig. 1). In most species, petals have three veins, but in a few species of the basal lineages of *Senna* and in *Cassia javanica*, petals have a single main vein. Also petals of the weedy and small flowered *S. uniflora* (clade V, fig. 1K) and the highly reduced upper petals in a few species of subclade VI (fig. 1M) have a single main vein, probably because of the reduction in petal size. Rarely, there appear to be two main veins in highly modified, asymmetric lower petals (figs. 1F, G) or two veins form a double strand in the standard petal (fig. 1H). The veins are particularly strong in highly concave and asymmetric lower petals (figs. 1F-H, J, M-P), and rarely also in the standard petal (figs. 1D, E).

Floral development and expression of floral asymmetry

The high diversity in patterns of floral asymmetry observed in *Senna*, in addition to monosymmetry, likely reflects diverse patterns of floral development. Floral development was previously known in detail from only one species, *S. didymobotrya* (Tucker 1996), a species of clade II with deflected carpel but monosymmetric androecium and corolla. We investigated the floral development of another species with moderately asymmetric flowers (*S. aciphylla*, fig. 6, clade IVa) and, for the first time, species with strongly asymmetric flowers (*S. wislizeni*, figs. 2H, 5, and *S. mollissima*, not shown, both clade III; *S. mucronifera*, figs. 2M, 7, clade IVb; *S. tonduzii*, figs. 3F, 8, clade VI). Flowers of *Senna* are subtended by a bract, while bracteoles appear to be absent (Tucker 1996). In fact, bracteoles may be initiated and then suppressed, as was found in

several papilionoids (Prenner 2004a) and in preliminary SEM studies we carried out in *Senna* species (unpublished data).

Organogenesis– In *S. didymobotrya*, the first sepal initiated is always median abaxial, and the other follow in spiral sequence (Tucker 1996). Petals are initiated almost simultaneously and also in spiral sequence, and petals develop equally. The organs of the outer androecial whorl are usually initiated after initiation of all petals. In both the outer and inner androecial whorl, the organs are initiated in unidirectional order, i.e. first the abaxial, and then the middle and adaxial organs. The carpel "is initiated as a central dome before any stamens" (Tucker 1996, p. 695). A nearly identical organogenesis to that of *S. didymobotrya* was found by Tucker (1996) in a cursory study of *S. artemisioides* (clade IVa), *S. bicapsularis* (clade VIIa), in *S. obtusifolia* (clade IVb), *S. occidentalis* (clade VIIa), and *S. surattensis* (clade IVa). Spiral sepal and petal initiation and unidirectional stamen initiation, as in *Senna*, corresponds to one of the two common patterns of organogenesis found in caesalpinioids. The other pattern is spiral sepal initiation and unidirectional petal and stamen initiation, and occurs in *Cassia* and *Chamaecrista*, for example (Tucker 1996). Although we did not focus on early stages, we found new aspects that add to the current knowledge of early development in *Senna* flowers. Sepal and petal initiation in our studied species are similar to that of *S. didymobotrya* (Tucker 1996). However, in *S. mucronifera, S. wislizeni, S. tonduzii* petal initials develop unequally, with the lower petals larger than the upper. In *S. aciphylla*, initials of upper petals are unequal, but they become of similar size in subsequent development. The organs of the outer androecial whorl are initiated after all petals in *S. wislizeni*, whereas their initiation overlaps with petal initiation in *S. aciphylla*. Unlike *S. didymobotrya*, in all species we studied, the carpel is initiated after the organs of the outer androecial whorl have been initiated, in *S. tonduzii*, even after the abaxial organs of the inner androecial whorl. Similar timing of carpel initiation as we observed in *Senna* appears to be present in *Cassia* and *Chamaecrista* (Tucker 1996).

Organ development – The sepals enlarge in the order of their spiral initiation (Tucker, 1996; this study). The petals become of equal size early (*S. didymobotrya*, Tucker 1996; *S. aciphylla*, this study) or they remain of different size up to the onset of petal overlapping (*S. auriculata*, *S. bicapsularis*, *S.* x *floribunda*, *S. obtusifolia*, Tucker, 1996) or up to anthesis (*S. mollissima, S. mucronifera, S. tonduzii, S. wislizeni*, this study). Two patterns of corolla aestivation occur in *Senna* (fig. 4D): a cochlear ascending pattern, as typical of most caesalpinioids (*S. auriculata, S. bicapsularis, S. corymbosa, S. lindheimeriana, S. multijuga, S. pallida, S. pendula*, and *S. surattensis*, Tucker, 1996), and a quincuncial pattern (*S. alata, S. artemisioides*, *S. didymobotrya*, *S. polyphylla, S. quinquangulata*, *S. racemosa*, Tucker, 1996; and *S. aciphylla, S. mollissima, S. mucronifera, S. wislizeni, S. tonduzii*; this study; see also fig. 4A-C). The two patterns are probably dependent on the speed of petal growth: early enlarging petals maintain the pattern of their spiral initiation resulting in quincuncial aestivation, whereas late enlarging petals are influenced by the developing floral monosymmetry, which results in a cochlear ascending aestivation. In early development, the median abaxial stamen enlarges faster than the other organs of the outer androecial whorl (*S. didymobotrya*, Tucker, 1996; *S. mucronifera, S. wislizeni, S. tonduzii*; this study), or almost as fast as the other organs of the same whorl (*S. aciphylla*, this study). In all species we studied, the middle and adaxial organs of the inner androecial whorl remain smaller than their counterpart of the outer whorl up to late-stage bud. In mid-stage bud, anthers begin to differentiate, and anther tips of abaxial stamens begin to elongate. The three abaxial stamens become of similar size and larger than the middle ones (*S. mucronifera, S. tonduzii*; this study), or the median abaxial one remains smaller than the lateral ones but is larger than the middle ones (*S. didymobotrya*; Tucker 1996). Size difference between the abaxial and middle stamens may appear only in late bud (*S. wislizeni*; this study). The carpel becomes arcuate and covered by hairs during mid-stage bud, and its stigmatic chamber is formed. The hairs fringing the stigmatic orifice develop in late bud.

Expression of floral asymmetry – Expression of floral asymmetry during development differs according to the pattern of asymmetry (see above). In enantiostylous *Senna* species, floral asymmetry involves either carpel deflection only, or also stamen deflection or modification in size, and petal modification in size and shape (tables 1, 2). Interestingly, flowers of all studied species with quincuncial corolla aestivation are enantiostylous. In contrast, those of most species with cochlear ascending corolla aestivation are monosymmetric. In general, ascending or descending cochlear patterns prevail in monosymmetric flowers of large orders of core eudicots, such as Fabales and Lamiales (Endress 1994). However, both patterns appear to occur also in *Chamaecrista* (Okpon 1969; Tucker 1996). More species should be studied in *Senna*, considering also species of the non-represented clades I and V, to test whether a quincuncial corolla aestivation is restricted to asymmetric, enantiostylous flowers.

Floral asymmetry appears at different developmental stages in the different floral whorls of *Senna*. Prominent corolla asymmetry is expressed in early bud; the lower petals become modified in shape and size when they begin to develop (figs. 5C, D, 7C, D, 8C, D). Asymmetry in the androecium is expressed in early mid-stage bud, when the size of the thecae in the median abaxial stamen becomes unequal (figs. 5F, 7F, 8G). In addition, in *S. tonduzii*, the androecium becomes twisted during anther differentiation (figs. 8G, I, J). Deflection of abaxial stamens to the side takes place only at anthesis. Asymmetry in the gynoecium of *S. tonduzii* appears to be expressed early in development; the carpel primordium appears to be displaced from the median plane of the flower (fig. 8E). In *S. aciphylla*, the ventral slit of the carpel is oriented slightly laterally in early bud (figs. 6E, F), as found in some other caesalpinioids with monosymmetric flowers (e.g., *Bauhinia malabarica*, Tucker, 1988; *Cassia javanica*, Tucker, 1996; *Ceratonia siliqua*, Tucker, 1992). It is not clear whether the early asymmetries in the gynoecium of *Senna* species are related with deflection of the carpel, which appears to occur in mid-stage bud (*S. aciphylla*, figs. 6H, I; *S. mucronifera*, figs. 7I, K, L; *S. tonduzii*, figs. 8H-L) or late bud (*S. didymobotrya*, Tucker, 1997; *S. wislizeni*, figs. 5I, L). In other enantiostylous families (mostly monocots), enantiostyly is expressed late in development: the style becomes deflected in late bud (*Wachendorfia paniculata*, *Dilatris corymbosa*, *Philydrum lanuginosum*) or only at anthesis (*Cyanella lutea*, *Monochoria australasica*, species of *Heteranthera*, and *Solanum rostratum*) (Jesson et al. 2003b). This is also the case in enantiostylous genera of Gesneriaceae (*Saintpaulia* and *Streptocarpus*, Harrison et al., 1999; Quentin C.B. Cronk, personal communication).

In caesalpinioids, floral symmetry varies from nearly polysymmetric to moderately or pronounced monosymmetric (Tucker 2003), and variously with asymmetric (*Senna*, *Chamaecrista* and *Labichea*; Tucker 1996, 1997, 1998; Marazzi et al. 2006). While in *Senna* most of the floral asymmetry is expressed in mid-stage bud or later, in *Chamaecrista fasciculata*, the entire organogenesis is asymmetric: the floral asymmetry in the androecium and corolla is expressed at early stages by precocious organ initiation on one side (left or right; Tucker 1996, 1999). Also in *Labichea lanceolata*, distantly related to *Senna* (Bruneau et al. 2001) and characterized by reduced number of floral organs and dissimilar stamens, floral asymmetry is expressed very early in development, including an asymmetric floral apex, and an asymmetric order of organ initiation (Tucker 1998). The asymmetrically curved or coiled keel of some papilionoids (Phaseolinae, Vicieae), is usually the result of late ontogeny (Tucker 1999), as shown for *Lathyrus latifolius* (Prenner 2003), and *Vigna caracalla* (Troll 1951; Prenner 2003). Also the androecium of several monosymmetric papilionoids is asymmetric in early developmental stages, because the adaxial antesepalous stamen is formed to the left or right of the median plane (Prenner 2004b).

Pollination biology and floral asymmetry evolution

Enantiostyly is restricted to buzz-pollinated flowers, in which other features, such as poricidal anther dehiscence, heteranthery and point-tipped stigmas evolved in relation to the unusual

pollination biology (e.g. Buchmann 1983). In *Senna*, pollen-collecting bees extract the pollen to feed their larvae by vibrating the middle 'feeding' stamens, which they clasp with their legs (e.g., Buchmann 1974; Delgado Salinas and Souza Sánchez 1977; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988, Westerkamp 2004). The abaxial 'pollinating' stamens are usually longer and display a higher diversity in anther dehiscence specialization than feeding stamens (Marazzi et al., submitted). Most species of *Senna* have a long and arcuate carpel with an extremely small, chambered or crater-like stigma (Marazzi et al., submitted). Dulberger et al. (1994) suggested that the diversity in inflection of the style tip and stigma position and orientation evolved in relation to the specific size and positioning of the bees visiting the flowers. However, also different lengths and inflections of the entire carpel may be associated with different size of the bees. Different directions of the carpel, i.e. straight or arcuate, median or deflected, may reflect different areas of the body of the bees which the stigma approaches or touches, with implications for the functional significance of enantiostyly (e.g., Jesson et al. 2003a; Jesson and Barrett 2005).

Enantiostyly has commonly been regarded as a device to promote outcrossing (Todd 1882), as pollen of a left floral morph is deposited on a place on the body of bees that corresponds to the position of the stigma in a right floral morph, and vice versa. However, in monomorphic enantiostylous taxa, such as *Senna*, the presence of both left and right floral morphs on the same plant and the observation that many of these plants are self-compatible, indicate that these floral asymmetries may actually promote geitonogamous self-pollination between different morphs. Other researchers have thus suggested that enantiostyly may facilitate the access of the pollen-collecting bees to the middle anthers, which the bees clasp and vibrate, forcing them to adopt a position that results in a greater pollen removal (Westerkamp 2004), and also protecting the gynoecium from damages by buzzing bees (Dulberger 1981; Dulberger et al. 1994). In contrast, Jesson et al. (2003a) and Jesson and Barrett (2005) provided evidence for the hypothesis that, compared to monosymmetry, enantiostyly functions to reduce geitonogamous pollen transfer with a concomitant increase in pollen export. Although these studies consider only style or carpel deflection, complex enantiostyly with asymmetric androecium and corolla, as found in *Senna*, may function in a similar way.

In *Senna*, different pointing directions of anther pores appear to be related with different directions of pollen release (Marazzi et al., submitted). Most anthers release pollen directly towards the floral center, and thus towards the bees. During buzzing, vibrational energy is transmitted from the thorax of the bee (clasping the middle stamens) to the flower and, thus, to other floral parts, such as the abaxial 'pollinating' stamens, the carpel and petals (Buchmann and Hurley 1978; Westerkamp 2004) or by the body of the bee touching the pollinating stamens (Endress 1997). In highly asymmetric flowers, anther pores of the abaxial pollinating stamens are usually directed towards the lower petals (Marazzi et al., submitted). These petals are positioned in such that pollen loss is avoided: they are highly concave, foot-shaped and/or asymmetric, and they partially surround the pollinating stamens (figs. 2F-H, L, M, 3D-L). Therefore, when bees buzz these flowers, pollen of the pollinating stamens is released towards the lower petals; vibration of these lower petals helps ricocheting the pollen flow, which finally adheres on the bees (e.g., Delgado Salinas and Souza Sánchez 1977; Westerkamp 2004). We observed that in species of clade II, with enantiostylous flowers and monosymmetric corolla, the standard petal is pronouncedly concave and partially encloses the floral reproductive organs (figs. 2C-E). In these flowers, the two large pollinating stamens are curved upwards, and their anther pores are directed towards the standard petal (Marazzi et al., submitted; figs. 2C-E). Therefore, the released pollen flow is likely ricocheted on the standard petal in a similar way, as described in flowers with concave and asymmetric lower petals. The particularly robust venation typical of both the highly concave standard petal and the asymmetric lower petals (figs. 1D, E, and figs. 1F-H, J, M-P, respectively), and the almost sessile shape, may be adaptations for transmitting more efficiently the vibrations produced by the buzzing bees from the middle stamens (on which the bees clasp), to the concave, ricocheting petal blade.

Although knowledge on the functional significance of enantiostyly allows us to suggest and interpret hypotheses of floral asymmetry evolution, detailed knowledge on the diverse floral morphology in *Senna* is a precondition to accurately infer hypotheses of floral asymmetry evolution. Floral symmetry is composed of several structural elements, as are pollination syndromes, and, since patterns of floral symmetry may not be homologous, the elements involved in the floral symmetry should be treated individually if used for phylogenetic hypotheses (Bruneau 1997; Herendeen et al. 2003) or optimization studies (Marazzi et al. 2006). Floral symmetry is particularly complex in *Senna*, because it does not include only monosymmetric flowers, but also several kinds of asymmetric flowers with different organs of different floral whorls contributing to the floral asymmetry. In addition, unrelated species have superficially similar asymmetric flowers (see table 1, 2).

Studies on the evolution of monomorphic and dimorphic enantiostyly in various angiosperm groups, suggested that style deflection in monomorphic enantiostyly evolved from an ancestral flower with a straight style in the median plane of the flower, and that the reciprocal positioning of the stamens evolved from a flower with a deflected style (Harrison et al. 1999; Jesson et al. 2003a; Jesson and Barret 2005). Preliminary analyses of ancestral character state reconstruction in *Senna*, using the molecular phylogenetic trees inferred by Marazzi et al. (2006) and the software Mesquite (Maddison and Maddison 2003), provide only a moderate support for the hypothesis that the carpel was not deflected in ancestral flowers of *Senna*, but a strong support that androecium and corolla were monosymmetric, and stamen deflection and asymmetric corolla evolved several times within the genus.

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TABLE 1

Clades of *Senna* and their patterns of floral symmetry (clades after Marazzi et al., 2006, submitted). Character states for symmetry of gynoecium (0, carpel in plane of floral monosymmetry; 1, carpel deflected to the side); androecium (0, organ not deflected nor modified in size; 1, organ deflected or modified in size); and corolla (0, petals not involved in floral asymmetry; 1, petals involved in floral asymmetry). Abbreviations used: C, concave; F, almost flat and open; lab, lateral abaxial stamen; LP oc, lower petal opposite to the deflected carpel; LP sc, lower petal same side of carpel; m, middle stamens; M, monosymmetric; mab, median abaxial stamen; N, not reduced; N°, not reduced and emarginate; R, reduced; SS, modified in size and shape; UP, upper petals. Asterisk indicates standard petal emarginate.

Clade	**Gynoec.**	**Androec.**		**Corolla**	**Petal shape**		
	carpel	**mab**	**lab**	**LP**	**LP oc**	**LP sc**	**UP**
I	0	0	0	0	F+M	F+M	N
II	1	0	0	0	F+M	F+M	N
IIIa	1	1	1	1	C+SS	C+SS	N
IIIb	1	0	0	1	C+SS	F+M	N
S. skinneri	1	1	0	1	C+M	F+M	N°
IVa	1	0	0	1	C+M	F+M	N
	1	0	1	1	C+M	F+M	N
IVb	1	0	0	0	F+M	F+M	N
	1	1	0	1	C+M	C/F+M	N
	1	1	0	1	C+SS	C+SS	N
IVb*	1	1	0	1	C+SS	C/F+M	N°
V	1	1	0	1	C+M	F+M	N°
VI (excl. VIa)	1	1	0	1	C+SS	C+M	R
	1	1	1	1	C+SS	F+M	N
	1	1	1	1	C+SS	C/F+M	R
	1	1	1	1	C+SS	F+SS	R
VIa	1	0	1	1	C+SS	C+M	N
VIIa	0	0	0	0	F+M	F+M	N°
	0	0	0	0	C+M	C+M	N°
VIIb	0	0	0	0	F+M	F+M	N°
Cassia	0	0	0	0	F+M	F+M	N

TABLE 2

Patterns of floral symmetry in *Senna*. Description of patterns with character states (0,1) of gynoecium, androecium and corolla, and petal shapes are based on table 1. Abbreviations used: C, concave; chr., character; F, almost flat and open; LP oc, lower petal opposite to the deflected carpel; LP sc, lower petal same side of carpel; M, monosymmetric; N, not reduced; N°, not reduced and emarginate; R, reduced; SS, modified in size and shape; UP, upper petals. In bold, taxa studied developmentally in this study, except *S. didymobotrya* studied by Tucker (1996).

	Pattern (chr. states)	**Petal shape LP oc & LP sc**	**Petal shape UP**	**Distribution in *Senna***	**Examples; figs.**
1	0 0 0 0	F+M	N	I	2A, B
		F+M	N°	VIIa, VIIb	3O, P
		C+M	N°	VIIa	2M
2	1 0 0 0	C+M	C+N	II	***S. didymobotrya***; 2C-E
		F+M	N	IVb	2O, P
3	1 0 0 1	C+M & F+M	N	Iva	***S. aciphylla***; **2I**
		C+SS & F+M	N	IIIb	2F
4	1 1 0 1	C+M & F+M	N°	*S. skinneri*, V	2K, 3A-C
		C+M & C/F+M	N	IVb	2N
		C+SS & F+M	N°	IVb*	*S. obtusifolia*
		C+SS & C+M	N°	IVb, IVb*	***S. mucronifera***; **2M**
		C+SS & C+M	R	VI (excl. VIa)	2F, 3H
		C+SS	N	IVb	*S. chloroclada*, 2L
5	1 0 1 1	C+M & F+M	N	IVa	2J
		C+SS & C+M	N	VIa	3J-L
6	1 1 1 1	C+SS & F+M	N	VI (excl. VIa)	3D, E
		C+SS & C/F+M	R	VI (excl. VIa)	3G, I
		C+SS & F+SS	R	VI (excl. VIa)	***S. tonduzii***; **3F**
		C+SS	N	IIIa	***S. wislizeni***; **2G**, H

APPENDIX

Taxa used in this study, source, and voucher information. Clades or subclades of *Senna* by Marazzi et al. (2006) and section by Irwin and Barneby (1982) are given in brackets (AS, *Astroites*; CH, *Chamaefistula*; PA, *Paradictyon*; PE, *Peiranisia*; PS, *Psilorhegma*; SE, *Senna*). Acronyms of herbaria or botanical gardens (in alphabetic order): CBG (=ANBG) = Australian National Botanic Gardens, BGB = Botanical Garden of the University of Basel, BGM = Botanischer Garten der Universität München, BGZ = Botanischer Garten der Universität Zürich, CTES = Instituto de Botánica del Nordeste, Corrientes, G = Conservatoire et Jardin Botaniques de la Ville de Genève, HUEFS = Universidad Estadual de Feira de Santana, MEXU = Universidad Nacional Autónoma de México, MT = Université de Montréal, PMA = Universidad de Panamá, PTGB = National Tropical Botanical Garden, Kauai, PY = Museo Nacional de Historia Natural de Paraguay, SI = Instituto de Botánica Darwinion, San Isidro, STRI = Smithsonian Tropical Research Institute, Balboa, Z = University of Zurich and Botanical Garden.

Taxon	**Clade**	**Section**	**Source**	**Voucher nr.**	**Locality and herbaria**
Cassia fistula L.	-	-	Cult.	*Endress 5007*	Australia, Queensland, Brisbane, Z
Cassia javanica L.	-	-	Cult.	*Endress 6411*	Australia, Queensland, Brisbane, Z
Senna aciphylla (Benth.) Randell	IVa	PS	Cult.	*CGB 9900061*	garden CBG, CBG, Z
S. acuruensis* var. *acuruensis (Benth.) H.S. Irwin & Barneby	VI	PE	Wild	*Conceição & Marazzi AC1125*	Brazil, Bahia, HUEFS, Z
S. alata (L.) Roxb.	II	SE	Wild	*Marazzi & al. BM026*	Paraguay, Caaguazú, PY, CTES, Z
S. aphylla (Cav.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM084*	Argentina, Santiago del Estero, CTES, Z
S. apiculata (M. Martens & Galeotti) H.S. Irwin & Barneby	VIIb	CH	Wild	*Marazzi & Flores BM170*	Mexico, Puebla, MEXU, Z
S. artemisioides (DC.) Randell	IVa	PS	Cult.	*Marazzi BM002*	garden BGZ s.n., Zurich, Z
S. atomaria (L.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM173*	Mexico, Oaxaca, MEXU, Z
S. aversiflora (Herbert) H.S. Irwin & Barneby	VI	PE	Wild	*Queiroz & Marazzi LQ 9204*	Brazil, Bahia, HUEFS, Z
S. birostris (Hook.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM090*	Argentina, Tucumán, CTES, Z
S. cana* var. *calva H.S. Irwin & Barneby	V	CH	Wild	*Conceiçao & Marazzi 1132*	Brazil, Bahia, HUEFS, Z
S. chacoënsis (L. Bravo) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM083*	Argentina, Santiago del Estero, CTES, Z
S. chloroclada (Harms) H.S. Irwin & Barneby	IVb	PE	Wild	*Marazzi et al. BM128*	Argentina, Salta, CTES, Z
S. didymobotrya (Fresen.) H.S. Irwin & Barneby	II	SE	Cult.	*Marazzi BM002*	garden BGZ 19700009, Z
S. hayesiana (Britton & Rose) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi & Alvárez BM150*	Republic of Panama, Panamá, PMA, STRI, Z
S. hirsuta* var. *leptocarpa (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	*Marazzi et al. BM065*	Paraguay, San Pedro, PY, CTES, Z
S. holwayana* var. *holwayana (Rose) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Flores BM161*	Mexico, Puebla, MEXU, Z

S. mollissima (Willd.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM181*	Mexico, Oaxaca, MEXU, Z
S. mucronifera (Benth.) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi et al. BM019*	Paraguay, Caaguazú, PY, CTES, Z
S. multijuga **var. *multijuga*** (Rich.) H.S. Irwin & Barneby	VI	PE	Cult.	*Marazzi & Alvárez BM151*	Republic of Panama, Panamá, PMA, STRI, Z
S. nicaraguensis (Benth.) H.S. Irwin & Barneby	II	SE	Wild	*Marazzi & Flores BM185*	Mexico, Chiapas, MEXU, Z
S. obtusifolia (L.) H.S. Irwin & Barneby	IVb	CH	Wild	*Marazzi et al. BM024*	Paraguay, Caaguazú, PY, CTES, Z
S. pallida (Vahl) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Flores BM178*	Mexico, Oaxaca, MEXU, Z
S. paradictyon (Vogel) H.S. Irwin & Barneby	II	PA	Wild	*Marazzi et al. BM028*	Paraguay, Alto Paraná, PY, CTES, Z
S. polyantha (Collad.) H.S. Irwin & Barneby	I	CH	Wild	*Marazzi & Flores BM172*	Mexico, Oaxaca, MEXU, Z
S. purpusii (Brandegee) H.S. Irwin & Barneby	VII	CH	Cult.	*Marazzi BM004*	garden BGB 3585/96-P, Z
S. quinquangulata (Rich.) H.S. Irwin & Barneby	IVb	CH	Wild	*Queiroz & Marazzi LQ 9220*	Brazil, Bahia, HUEFS, Z
S. rigida (Hieron.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi et al. BM108*	Argentina, Salta, CTES, Z
S. robiniifolia (Benth.) H.S. Irwin & Barneby	VI	PE	Cult.	*Marazzi BM005*	garden BGM 98/3500w, Z
S. rugosa (G. Don) H.S. Irwin & Barneby	IVb	CH	Wild	*Queiroz & Marazzi LQ 9181*	Brazil, Bahia, HUEFS
S. septemtrionalis (Viviani) H.S. Irwin & Barneby	VIIa	CH	Cult.	*Marazzi BM140*	garden BGM s.n., Z
S. silvestris **var. *guaranitica*** (Chodat & Hassl.) H.S. Irwin & Barneby	I	CH	Wild	*Marazzi et al. BM068*	Paraguay, San Pedro, PY, CTES, Z
S. skinneri (Benth.) H.S. Irwin & Barneby	IV	CH	Wild	*Marazzi & Flores BM176*	Mexico, Oaxaca, MEXU, Z
S. spectabilis (DC.) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi et al. BM029*	Paraguay, Alto Paraná, PY, CTES, Z
S. tonduzii (Standl.) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Flores BM187*	Mexico, Chiapas, MEXU, Z
S. uniflora (Mill.) H.S. Irwin & Barneby	V	CH	Wild	*Marazzi & Flores BM186*	Mexico, Chiapas, MEXU, Z
S. unijuga (Rose) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM167*	Mexico, Puebla, MEXU, Z
S. villosa (Mill.) H.S. Irwin & Barneby	VIIb	VS	Wild	*Marazzi & Flores BM174*	Mexico, Oaxaca, MEXU, Z
S. williamsii (Britton & Rose) H.S. Irwin & Barneby	VI	PE	Wild	*Marazzi & Alvárez BM158*	Republic of Panama, Panamá, PMA, STRI, Z
S. wislizeni (A. Gray) H.S. Irwin & Barneby	III	PE	Wild	*Marazzi & Flores BM169*	Mexico, Puebla, MEXU, Z.

Fig. 1 Petal diversity in species of major clades and subclades of *Senna* and of *Cassia javanica*.

A, *Cassia javanica*; B, *Senna polyantha* (I); C, *S. silvestris* var. *guaranitica* (I); D, *S. paradictyon* (II); E, *S. didymobotrya* (II); F, *S. unijuga* (III); G, *S. wislizeni* (III); H, *S. mollissima* (III), I, *S. aciphylla* (IVa); J, *S. mucronifera* (IVb); K, *S. uniflora* (V); L, *S. cana* var. *calva* (V); M, *S. acuruensis* (VI); N, *S. tonduzii* (VI); O, *S. pallida* (VI); P, *S. chacoënsis* (VIa); Q, *S. villosa* (VIIb); R, *S. hirsuta* (VIIa). Some petals torn during flattening process (arrows). Dotted lines indicate missing parts of petal tissue. Scale bar as indicated.

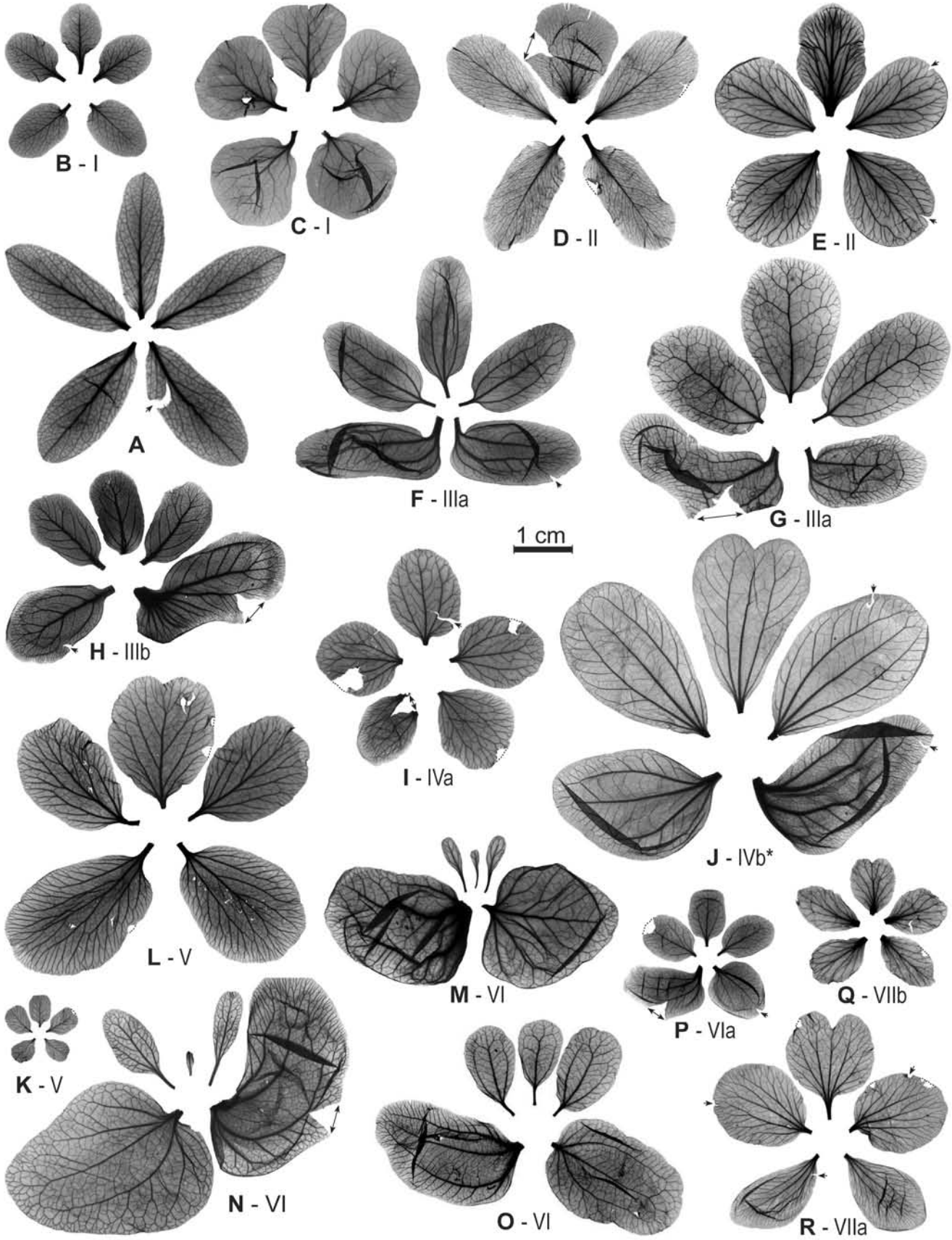
B - I
C - I
D - II
E - II
A
F - IIIa
G - IIIa
1 cm
H - IIIb
I - IVa
J - IVb*
L - V
M - VI
P - VIa
Q - VIIb
K - V
N - VI
O - VI
R - VIIa

Fig. 2 Flowers of *Senna* species of clades I-IV.

A, *S. polyantha* (I); B, *S. silvestris* var. *guaranitica* (I); C, *S. nicaraguensis* (II); D, *S. martiana* (II); E, *S. paradictyon* (II); F, *S. atomaria* (III); G, *S. unijuga* (III); H, *S. wislizeni* (III), I, *S. aciphylla* (IVa); J, *S. artemisioides* (IVa); K, *S. skinneri* (IV); L, *S. chloroclada* (IVb); M, *S. mucronifera* (IVb); N, *S. rugosa* (IVb); O, *S. hayesiana* (IVb); P, *S. quinquangulata* (IVb). For flower size see Irwin and Barneby (1982).

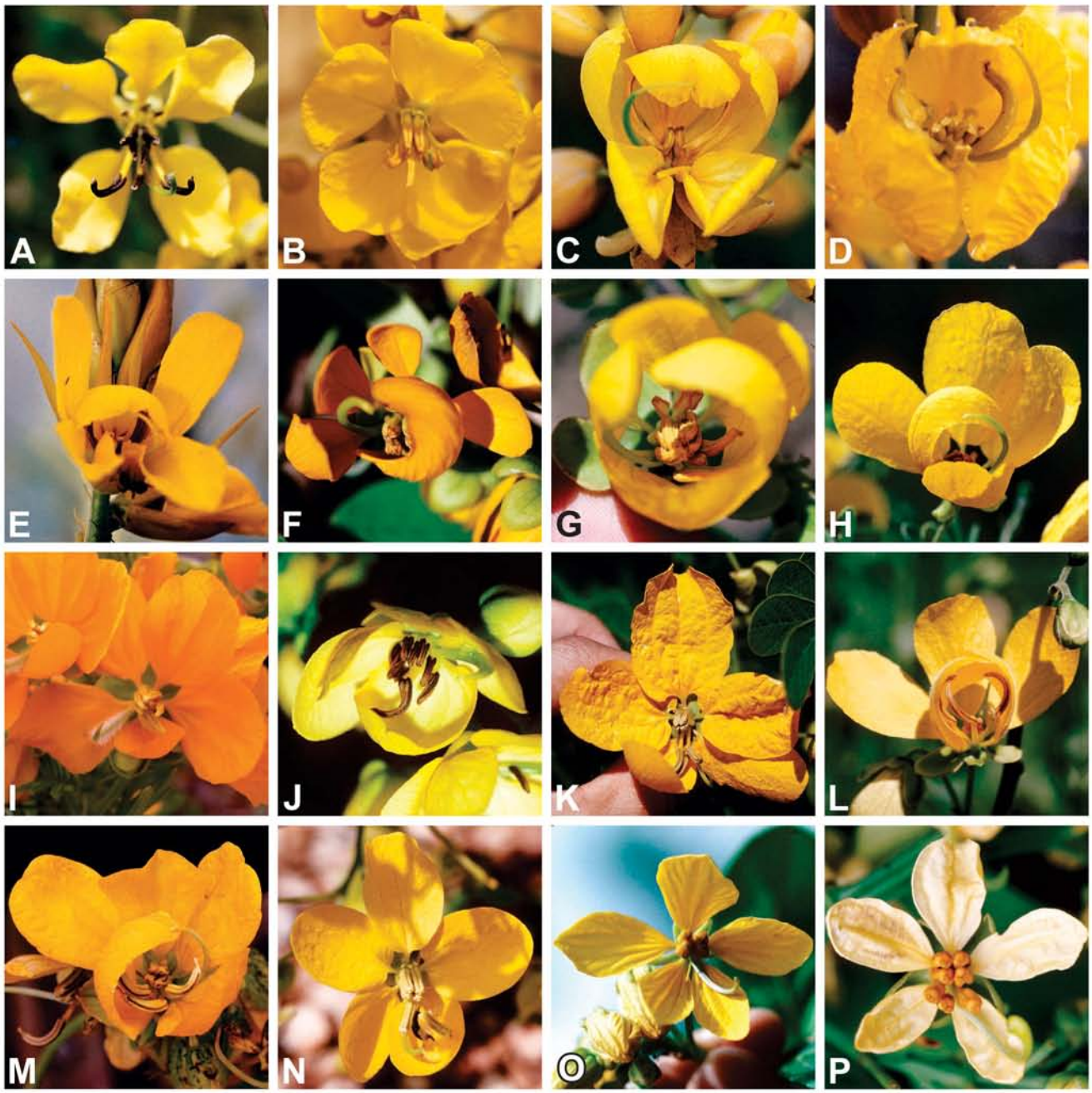
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Fig. 3 Flowers of *Senna* species of clades V-VII.

A, *S. cana* var. *calva* (V); B, *S. uniflora* (V); C, *S.* cf. *velutina* (V; photograph by M. Belgrano); D, *S. aversiflora* (VI); E, *S. holwayana* (VI); F, *S. tonduzii* (VI); G, *S. acuruensis* var. *acuruensis* (VI); H, *S. multijuga* var. *multijuga* (VI), I, *S. pallida* (VI); J, *S. aphylla* (VIa); K, *S. chacoënsis* (VIa); L, *S. rigida* (VIa); M, *S. hirsuta* (VIIa); N, *S. septemptrionalis* (VIIa); O, *S. subulata* (VIIa; photograph by M. Belgrano); P, *S. villosa* (VIIb). For flower size see Irwin and Barneby (1982).

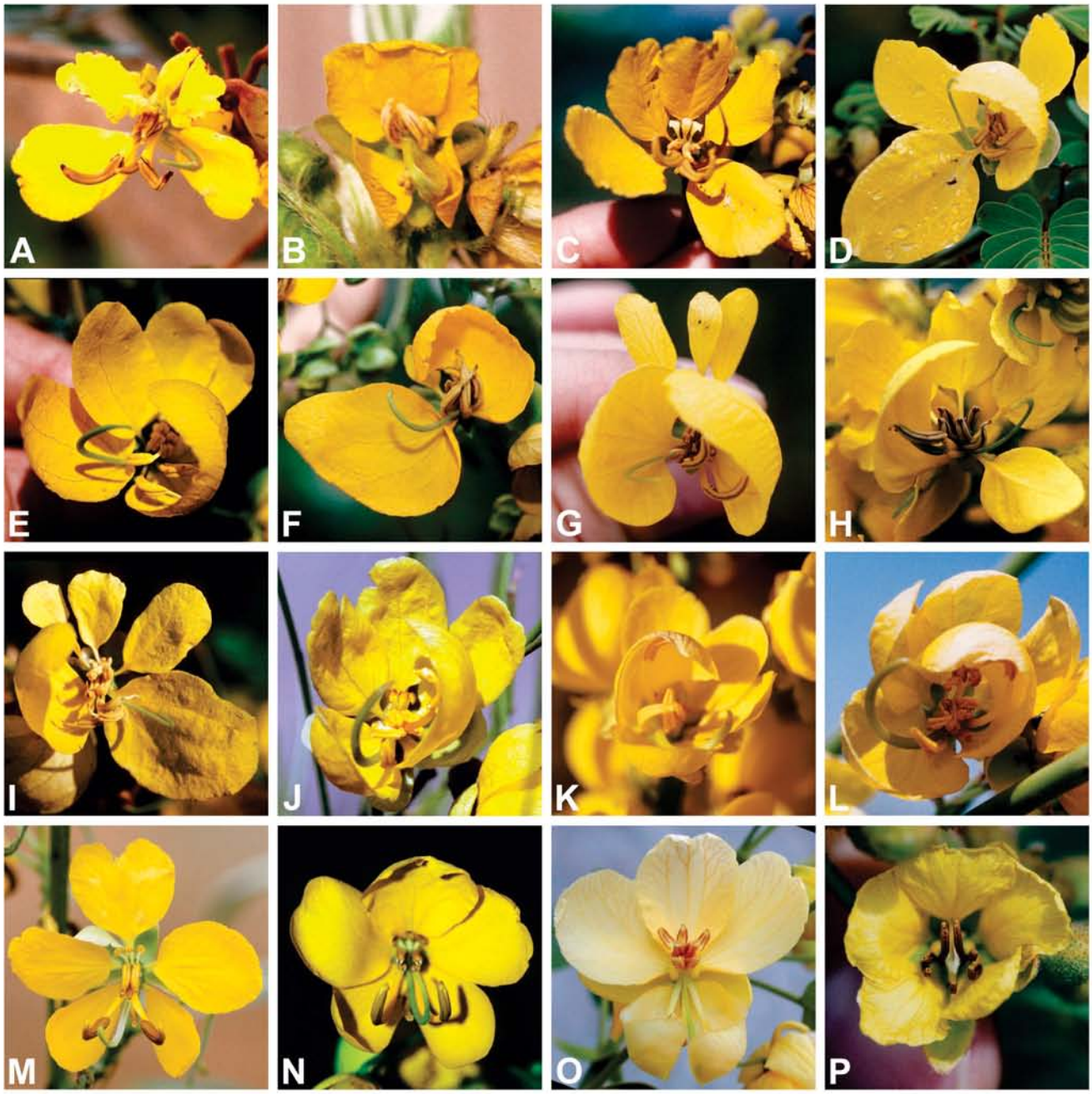
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Fig. 4 Floral diagram of *Senna*, transverse sections (TS) of floral buds of species studied developmentally, and corolla aestivation.

All TS and floral diagrams represent right floral morphs (clockwise calyx aestivation indicated by arrow in floral diagram). A, *S. wislizeni* (clade III); three adaxial staminodes. B, *S. aciphylla* (clade IVa); all stamens fertile. C, *S. tonduzii* (clade VI); level of TS in bud above the highly reduced adaxial staminodes and upper petals. D, patterns of corolla aestivation. For clarity, middle stamens are indicated on all TS. Abbreviations: ab, abaxial stamen; ad, adaxial stamen; m, middle stamen; LP, lower petals; S, sepals; UP, upper petals. Asterisks indicate gynoecium. Scale bars = 1 cm.

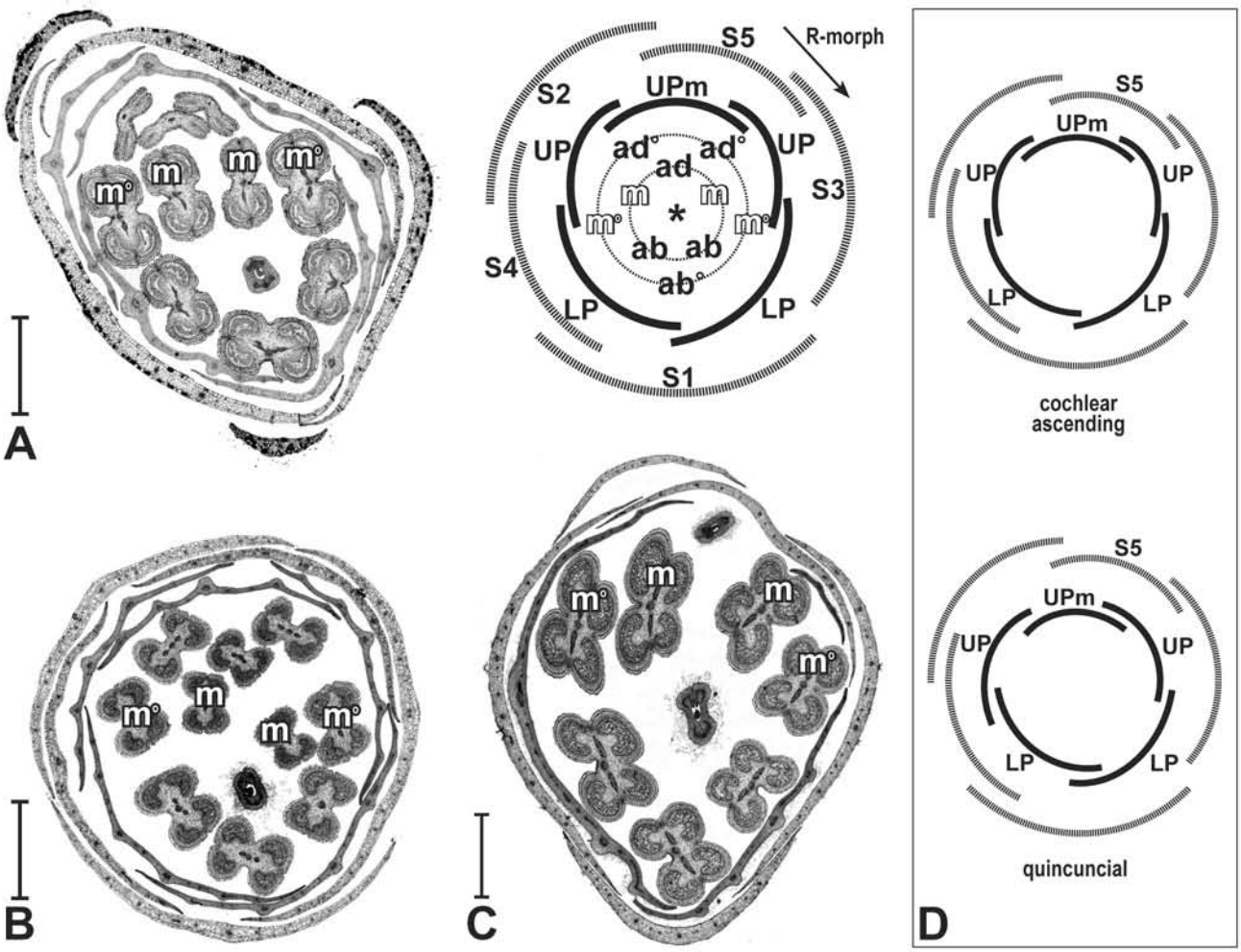
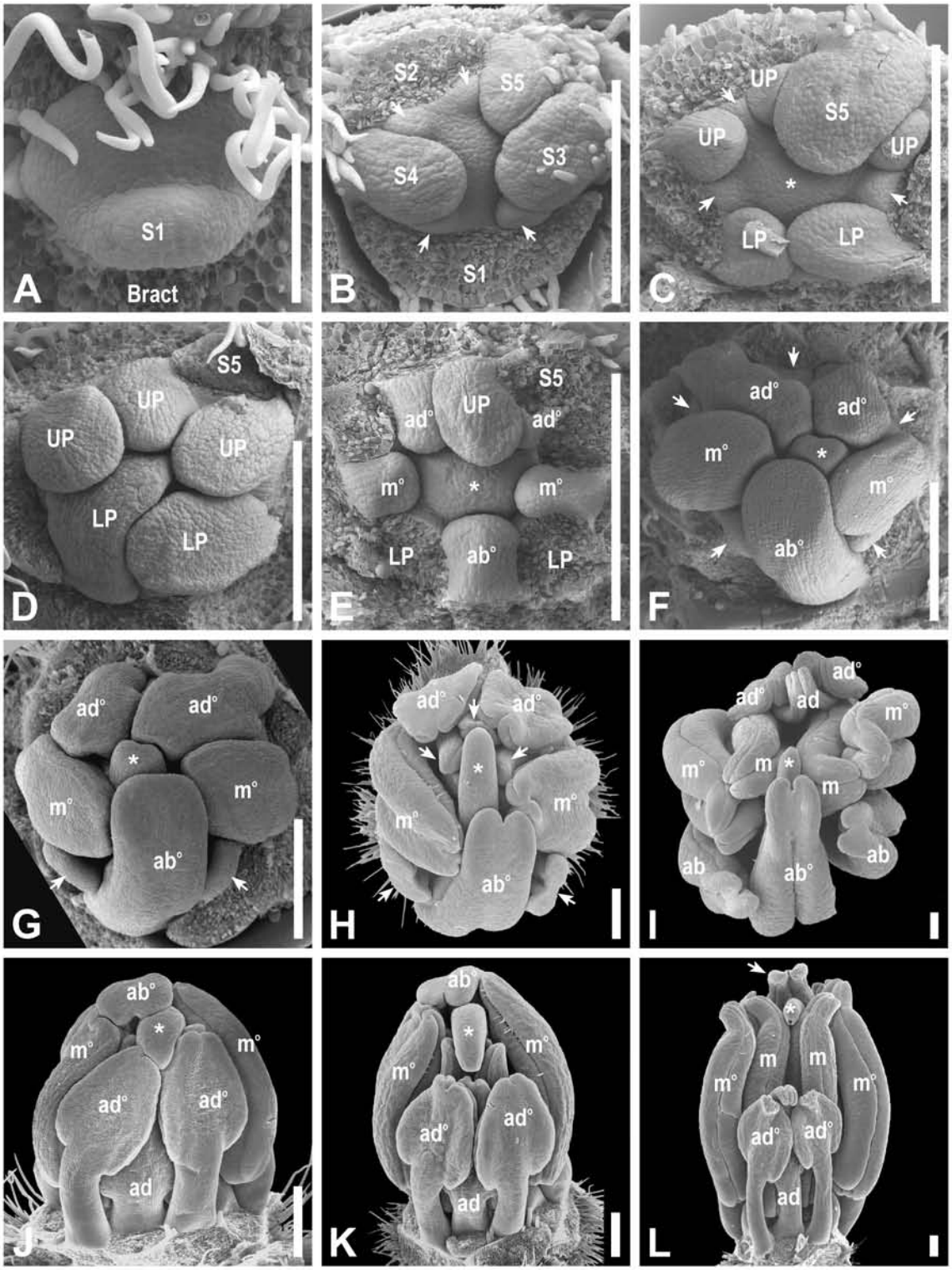

A
m°
m
m
m°
S5
R-morph
S2
UPm
UP
ad°
ad°
UP
ad
S3
m
m
m°
*
m°
ab
ab
S4
ab°
LP
LP
S1
S5
UPm
UP
UP
LP
LP
cochlear
ascending
B
m°
m
m
m°
C
m°
m
m
m°
S5
UPm
UP
UP
LP
LP
quincuncial
D

Fig. 5 Floral development of *Senna wislizeni* (clade III).

Subtending bract removed in all flowers; one to all sepals removed in B-L; one to all petals removed in D-L. Flowers oriented with adaxial side upwards in A-I. Right floral morph in B-F, left floral morph in G-L. **A**, initiation of first sepal (S1). **B**, quincuncial aestivation of calyx (S1-S5), initiation of petals (arrows). **C**, unequal development of lower petals (LP); initiation of organs of outer androecial whorl (arrows). **D**, quincuncial corolla aestivation; shape differentiation of one (left) lower petal. **E**, outer androecial whorl and carpel. **F**, initiation of organs of inner androecial whorl (arrows). **G**, differentiation of organs of outer androecial whorl into adaxial staminodes (ad°), middle stamens (m°) and abaxial stamens (ab°); unequal development of lateral abaxial stamens of inner androecial whorl (arrows) vs. other organs of the same whorl. **H**, differentiation of anther tips and lateral furrows; organs of inner androecial whorl partially hidden (arrows); carpel arcuate and in median plane of floral symmetry. **I**, anther differentiation in mid-stage bud; carpel slightly deflected. **J**, adaxial view of G. **K**, adaxial view of H. **L**, adaxial view of I; late stage of anther development, with the median abaxial stamen (arrow) largest; orifice of stigmatic chamber below asterisk. Asterisk indicates gynoecium. Scale bars in A = 125 µm, in B-L = 250 µm.


A
S1
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S5
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S1
C
UP
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Fig. 6 Floral development of *Senna aciphylla* (clade IVa).

Subtending bract removed in all flowers; four or all sepals removed in C-L; all petals removed in F-L. Flowers oriented with adaxial side upwards in A-I. Right floral morph in A, E, left floral morph in B-D, F-L. **A**, spiral sequence of first three sepals (S1-S3) and initiation of fourth and fifth sepals (arrows). **B**, quincuncial aestivation of calyx (S1-S5). **C**, development of lower petals (LP) and one lateral upper petal (on the right), and initiation of other upper petals; initiation of four stamens of outer androecial whorl (arrows). **D**, unequal development of petals; development of stamens of outer androecial whorl (ab°, m°, ad°) and abaxial organs of inner whorl (arrows); androecium nearly monosymmetric; initiation of carpel (asterisk). **E**, quincuncial corolla aestivation; oblique direction of ventral slit of carpel (asterisk). **F**, differentiation of stamens of outer androecial whorl; development of abaxial organs of inner whorl (ab). **G**, differentiation of stamens of outer androecial whorl and of abaxial organs of inner whorl. **H**, differentiation of anther tips and lateral furrows in mid-stage bud; organs of inner androecial whorl partially hidden (arrows); androecium nearly monosymmetric; carpel arcuate and laterally deflected. **I**, late stage of anther differentiation; carpel laterally deflected. **J**, adaxial view of G. **K**, adaxial view of H. **L**, lateral/adaxial view of I. Scale bars = 250 µm.

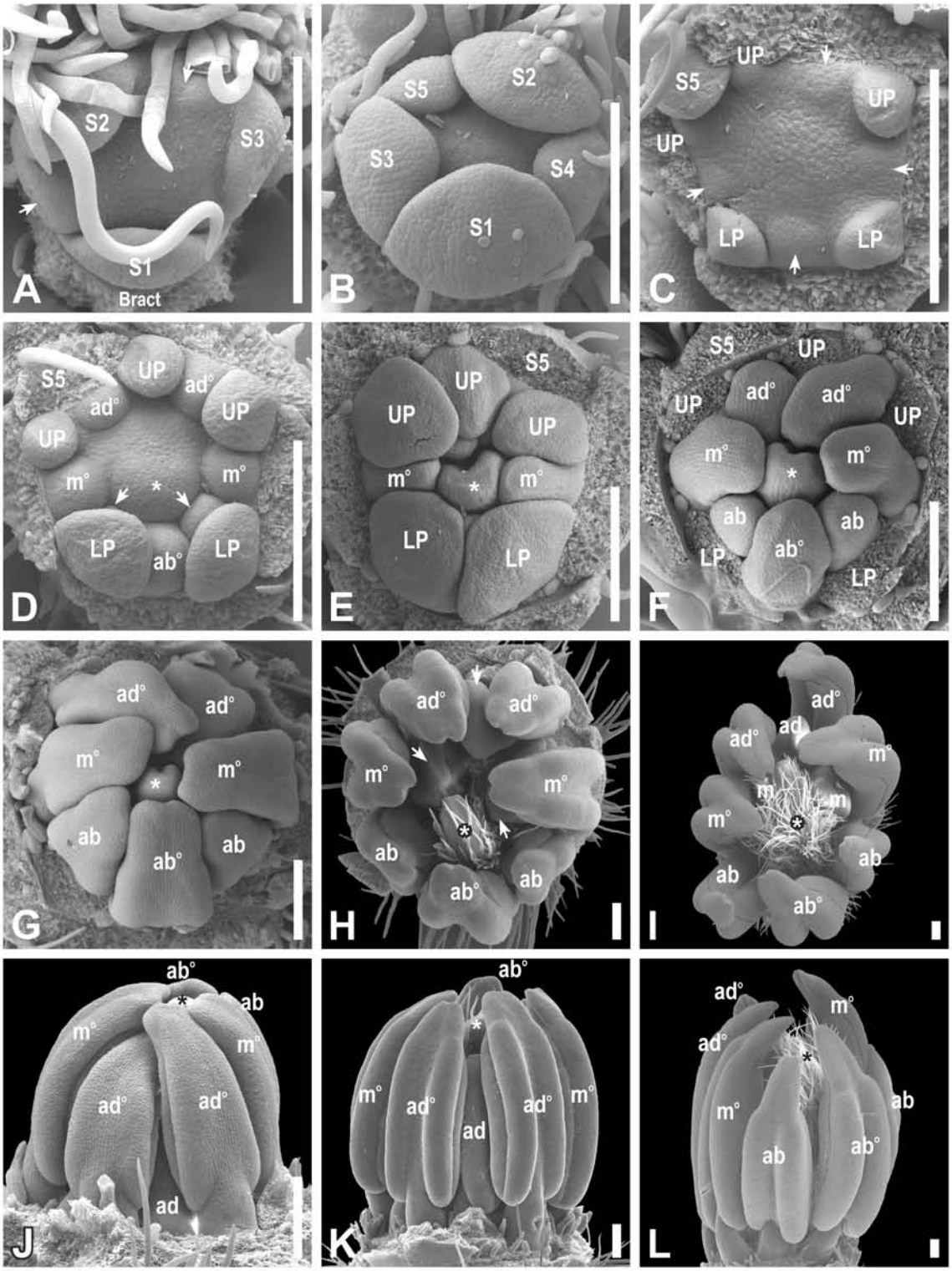
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Fig. 7 Floral development of *Senna mucronifera* (clade IVb).

Subtending bract removed in all flowers; two to all sepals removed in B-L; one to all petals removed in D, F-L. Flowers oriented with adaxial side upwards in A-I. Right floral morph in C, D, F, G, I, J, L, left floral morph in A, B, E, H, K. **A**, initiation of first and second sepal (S1, S2). **B**, quincuncial calyx aestivation (S1-S5), initiation of petals (arrows). **C**, unequal differentiation of lower petals (LP) vs. upper petals (UP); initiation of organs of outer androecial whorl (arrows). **D**, shape differentiation of one lower petal into foot shaped organ; development of abaxial organ of the inner androecial whorl (arrow). **E**, quincuncial corolla aestivation. **F**, development of organs of both androecial whorls (ab°, ab, m°, m, ad°, arrow); development of carpel (asterisk). **G**, differentiation of organs of androecial whorls into adaxial staminodes (ad°, median arrow), middle stamens (m°, lateral arrows) and abaxial stamens (ab°, ab); carpel arcuate. **H**, differentiation of anther tips and lateral furrows; unequal growth of middle and adaxial organs vs. abaxial organs of inner androecial whorl (arrows). **I**, anther differentiation in late mid-stage bud; carpel slightly deflected. **J**, adaxial view of G. **K**, adaxial view of H; orifice of stigmatic chamber below asterisk. **L**, adaxial view of I. Scale bars in A = 125 µm, in B-L = 250 µm.

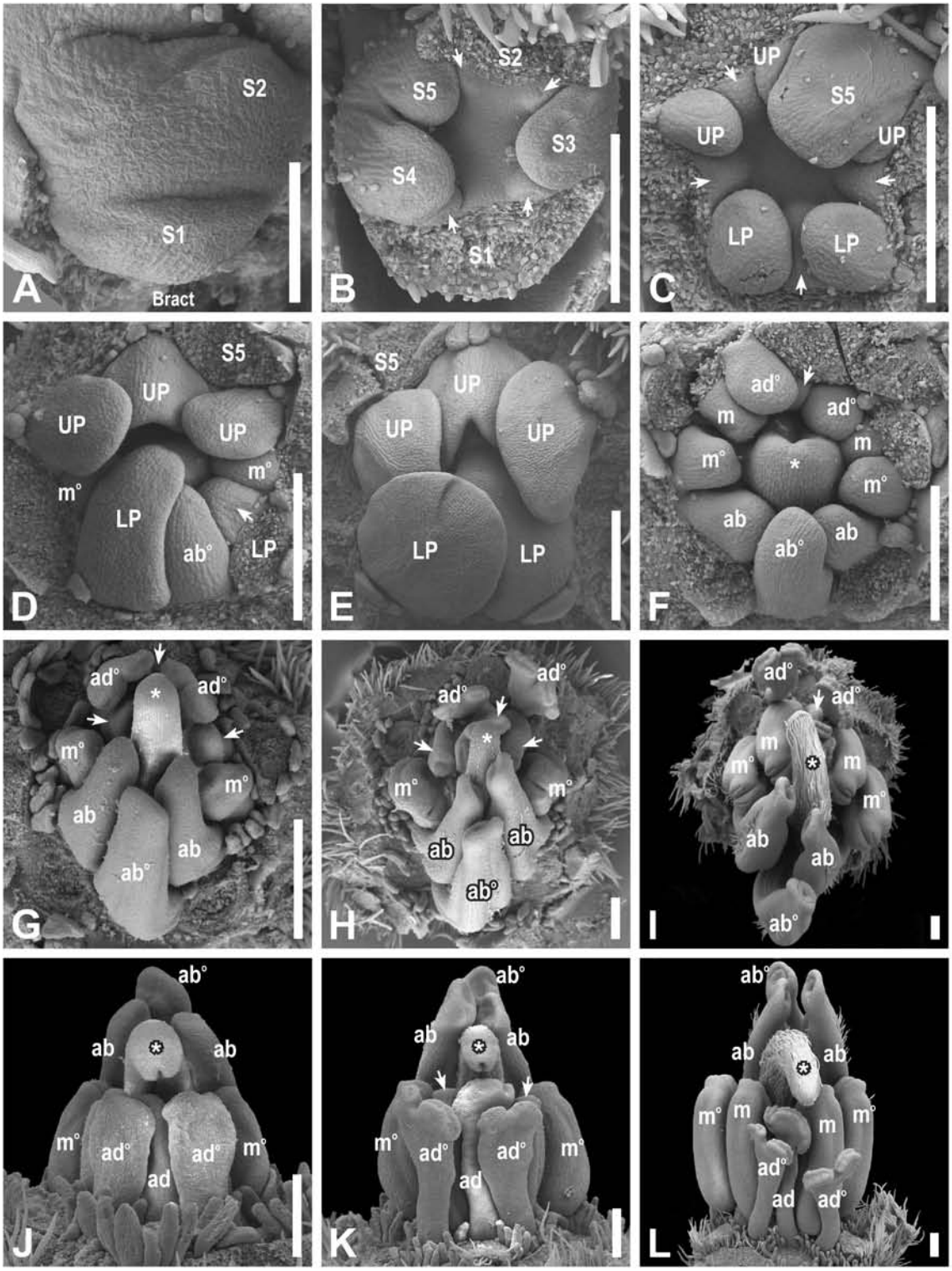
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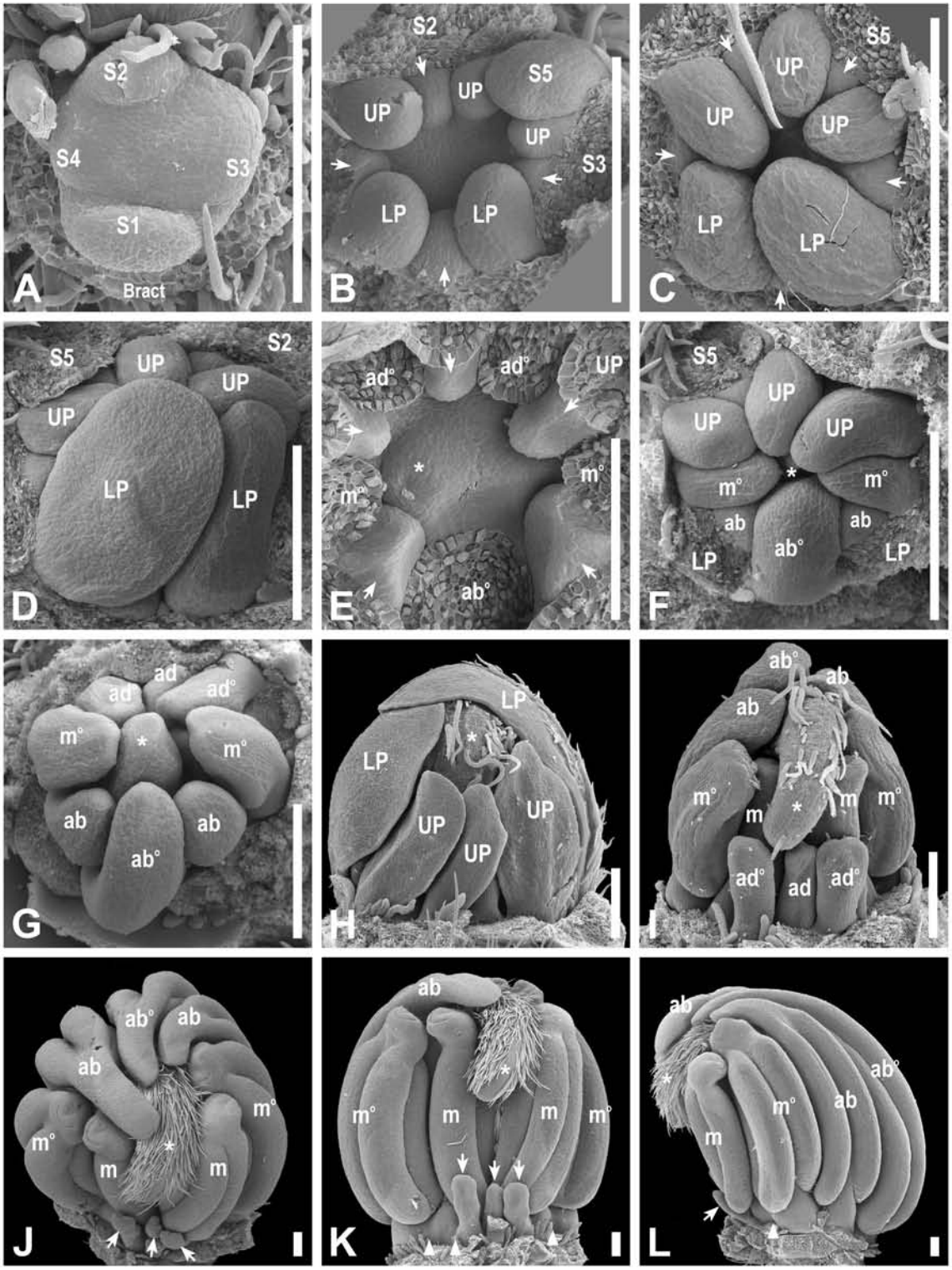
Fig. 8 Floral development of *Senna tonduzii* (clade VI).

Subtending bract removed in all flowers; four or all sepals removed in B-L; three to all petals removed in E-G, I-L. Flowers oriented with adaxial side upwards in A-I. Right floral morph in A-C, left floral morph in D-L. **A**, spiral initiation of first four sepals (S1- S4). **B**, unequal development of lower (LP) and upper petals (UP); organs of outer androecial whorl initiated (arrows). **C**, shape differentiation in lower petals; development of organs of outer androecial whorl (arrows). **D**, corolla aestivation. **E**, beginning of development of organs of inner androecial whorl (arrows); carpel initiation (asterisk). **F**, development of organs of both androecial whorls (ab°, ab, m°) and of upper petals; development of carpel (asterisk). **G**, differentiation of organs of androecial whorls into adaxial staminodes (ad°, ad), middle stamens (m°) and abaxial stamens (ab°, ab). **H**, unequal differentiation of upper vs. lower petals. **I**, early mid-stage of differentiation of anther tips and lateral furrows; carpel deflected. **J,** late stage of anther differentiation. Staminodes indicated by arrows; carpel strongly deflected. **K**, adaxial view of J; filament union indicated by arrowheads. **L**, lateral view of J; filament union indicated by arrowheads. Scale bars: in A-D, G-L = 250 µm, in E = 125 µm.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GENERAL SUMMARY

The three parts that compose this thesis are related to various aspects of floral structural specialization and evolution in association with a specialized pollination mode. The genus *Senna* represents an ideal study group to focus on these aspects.

In **part 1**, phylogenetic relationships within the large, diverse and widespread genus *Senna* based on parsimony analyses of three chloroplast-DNA regions (*rpS*16, *rpL*16, and *matK*) are clarified. The results support the monophyly of only one, *Psilorhegma*, of the four currently recognized non-monotypic sections, while *Chamaefistula*, *Peiranisia*, and *Senna* are paraphyletic, and the monotypic *Astroites* and *Paradictyon* are nested within two of the seven major clades identified by our molecular phylogeny. Two clades (I, VII) include only species with monosymmetric flowers, while the remaining clades (II–VI) contain species with asymmetric, enantiostylous flowers, in which either the gynoecium alone or, in addition, corolla and androecium variously contribute to the asymmetry. Results further suggest that flowers were ancestrally monosymmetric with seven fertile stamens and three adaxial staminodes, later switched to asymmetry, and reverted to monosymmetry in clade VII. Fertility of all 10 stamens is a derived state, characterizing the *Psilorhegma* subclade. Therefore, neither the current classification nor the traditional hypotheses of floral evolution within *Senna* were supported by the molecular phylogenetic analyses. In this part, I also explore the evolution of extrafloral nectaries. They evolved once, constituting a synapomorphy for clades IV–VII, and may represent a key innovation in plant defense strategies that enabled *Senna* to undergo large-scale diversification.

In **part 2**, the diversity of floral elements not involved in the symmetry of *Senna* flowers, including in particular floral structural specializations associated with buzz pollination, which are expressed in the androecium and gynoecium is investigated. I studied the floral structure of 69 species from all major clades of *Senna*, focusing on heteranthery, anther dehiscence, pore position, extension of the lateral furrow of the thecae, cell wall thickening in the anther tip, and stigma diversity. Filament union is reported for the first time in the genus. The union involves the seven adaxial androecial organs, a pattern unique in legumes. My investigations identified novel morphological characteristics that are congruent with the clades supported by the molecular phylogenetic studies. Anthers of abaxial stamens with the least differentiated dehiscence pattern, i.e., two separate pores and separate thecae, are found in most major clades (I, III-V, VII). Anthers with apically confluent thecae, forming a shared chamber, and/or with a single pore by confluence of two pores, represent specialized patterns (clades II, IV, VI, VII). Diverse anther tips may reflect different strategies of pollen dispersal; anther pore position may influence pollen flow direction. Anther tip elongation in the abaxial stamens and constriction between the thecae and the anther tip may influence the speed and/or amount of the released pollen.

In **part 3**, the floral structural elements involved in the floral asymmetry of *Senna* are identified. To assess the homology of the diverse patterns of floral asymmetry observed in *Senna*, the flower structure of 39 species from all clades of *Senna* (plus two species of *Cassia*, sister genus of *Senna*) and the development of four enantiomorphic species from different clades were studied . I found that the asymmetry morph of a flower is correlated with the direction of the spiral calyx aestivation: clockwise direction corresponds to a right morph, while counter-clockwise direction corresponds to a left morph. Five major patterns of floral asymmetry ere recognized, resulting from different combinations of at least six structural elements: (1) deflection of the carpel; (2) deflection of the median abaxial stamens; (3) deflection of one lateral abaxial stamen or, rarely, (4) modification in size of this stamen; and modification in shape and size of (5) one or (6) both lower petals. Petals have diverse venation: most *Senna* species have three main petal veins, a few species of the basal lineages of *Senna*, and also *Cassia*, have only one main vein. Expression of floral asymmetry during development differs among floral whorls. Prominent

corolla asymmetry begins in early bud (unequal development of lower petals). Androecium asymmetry begins in mid-stage bud (unequal development of thecae in the median abaxial stamen; twisting of the androecium) or at anthesis (stamen deflection). Gynoecium asymmetry begins in early bud (primordium appearing off the median plane; ventral slit laterally oriented) or mid-stage to late bud (carpel deflection). In both monosymmetric and highly asymmetric corollas of enantiostylous flowers, pronouncedly concave and robust petals likely function to ricochet pollen flow and to reduce loss of pollen during buzz pollination. Finally, preliminary ancestral character state reconstructions moderately support the hypothesis that the gynoecium was not deflected in ancestral *Senna* flowers, but strongly support that asymmetric androecium and corolla evolved from enantiostylous flowers.

ZUSAMMENFASSUNG

Die drei Teile der vorliegenden Dissertation befassen sich mit verschiedenen Aspekten der strukturellen Blütenspezialisierung und -evolution in Verbindung mit einer spezialisierten Bestäubungsbiologie. Die artenreiche Gattung *Senna* (Leguminosen) bildet eine ideale Gruppe zur Untersuchung dieser Aspekte.

In **Teil 1** werden phylogenetische Beziehungen basierend auf Parsimony-Analysen von drei Chloroplasten-DNA-Regionen (*rpS*16, *rpL*16, and *matK*) aufgeklärt. Die Resultate unterstützen die Monophylie der Sektion *Psilorhegma*, einer der vier gegenwärtig anerkannten nicht-monotypischen Sektionen, während *Chamaefistula*, *Peiranisia*, und *Senna* paraphyletisch sind, und die monotypischen *Astroites* and *Paradictyon* in zwei der Hauptclades eingebettet sind. Zwei Clades (I, VII) enthalten nur Arten mit monosymmetrischen Blüten. Die übrigen Clades (II-VI) enthalten Arten mit asymmetrischen, enantiostylen Blüten, bei denen entweder nur das Gynoecium, oder auch die Krone und das Androecium vielfältig an der Blütenasymmetrie beteiligt sind. Die molekular-phylogenetischen Ergebnisse weisen auch darauf hin, dass ursprüngliche Blüten bei *Senna* monosymmetrisch waren mit sieben fertilen Staubblättern und drei adaxialen Staminodien, dass asymmetrische Formen davon abgeleitet sind, und dass im Clade VII sekundär Monosymmetrie evoluiert ist. Fertilität aller 10 Staubblätter ist innerhalb der Gattung ein abgeleiteter Merkmalszustand, der für den *Psilorhegma*-Clade charakteristisch ist. Somit werden weder die traditionelle Klassifikation noch traditionelle Hypothesen über die Blütenevolution bei *Senna* von den molekular-phylogenetischen Analysen unterstützt. In Teil 1 wird auch die Evolution von extrafloralen Nektarien untersucht, welche nur einmal entstanden sind, und eine Synapomorphie für die Clades IV-VII darstellen. Sie sind möglicherweise innerhalb der Gattung eine Schlüsseleigenschaft, welche es *Senna* als Abwehrstrategie gegen Herbivoren ermöglicht hat, sich stark zu differenzieren.

In **Teil 2** wird die Vielfalt der Blütenelemente erforscht, die nicht direkt an der Blütensymmetrie beteiligt sind, vor allem die mit der Buzz-Bestäubung verbundenen strukturellen Blütenspezialisierungen, welche besonders im Androecium und Gynoecium ausgeprägt sind. Die Blütenstruktur von 69 Arten aller Hauptclades von *Senna* wurde untersucht, insbesondere Heterantherie, Antherendehiszenz, Lage der Antherenporen, Ausdehnung der Stomien in den Theken, Zellwandverdickungen in der Antherenspitze, und Stigmaform. Eine Vereinigung der Filamente wurde erstmals festgestellt in der Gattung. Die sieben adaxialen Androeciumorgane sind kongenital verwachsen, was einzigartig ist in der Familie der Leguminosae. Neue morphologische Eigenschaften, die mit den Clades der molekularen Phylogenie übereinstimmen, wurden gefunden. Abaxiale Antheren mit dem am wenigstens differenzierten Muster (d.h. zwei getrennte Poren und getrennte Theken) kommen in den meisten Hauptclades (I, III-V, VII) vor. Antheren des spezialisierten Musters haben apikal zusammenfliessende Theken, die eine gemeinsame Kammer bilden, und/oder eine einzige Pore aus zwei vereinigten Poren (Clades II, IV, VI, VII). Diverse Ausrichtungen der Antherenspitzen widerspiegeln möglicherweise verschiedene Pollenverbreitungsmuster mit verschiedenen Zielrichtungen des Pollenstrahls. Eine Verlängerung der Antherenspitze in den abaxialen Staubblättern und eine Einschnürung zwischen den Theken und der Antherenspitze könnte die Geschwindigkeit und/oder die Menge des abgegebenen Pollens beeinflussen.

In **Teil 3** werden die strukturellen Blütenelemente identifiziert, die an der Blütenasymmetrie beteiligt sind. Um die Homologie der bei *Senna* vorkommenden vielfältigen Mustern der Blütenasymmetrie festzustellen, wurde die Blütenstruktur von 39 Arten aller Hauptclades von *Senna* (plus zwei Arten von *Cassia*, der Schwestergattung von *Senna*) und die Blütenentwicklung von vier enantiomorphen Arten aus verschiedenen Clades untersucht. Es wurde gefunden, dass die Enantiomorphie der Blüten mit der Richtung der spiraligen

Kelchästivation korreliert ist: eine Richtung im Uhrzeigersinn entspricht einem rechten Morph, eine Richtung gegen den Uhrzeigersinn entspricht einem linken Morph. Fünf Hauptmuster der Blütenasymmetrie wurden unterschieden, welche aus verschiedenen Kombinationen von mindestens sechs strukturellen Elementen enstehen: (1) Krümmung des Gynoeciums aus der Medianebene; (2) Krümmung des medianen abaxialen Staubblattes aus der Medianebene; (3) Krümmung eines lateralen abaxialen Staubblattes, oder selten (4) Vergrösserung desselben; Grössen- und Gestaltveränderung von (5) einem oder (6) beiden unteren Kronblättern. Kronblätter haben verschiedene Nervaturmuster: Die meisten *Senna*-Arten haben drei Hauptleitbündel, und einige wenige Arten der basalen Verwandschaftskreise von *Senna* plus *Cassia* haben nur ein Hauptleitbündel. Die Ausprägung der Blütenasymmetrie während der Entwicklung ist zwischen den Blütenwirteln verschieden. Starke Asymmetrie der Krone beginnt bereits in frühen Stadien. Asymmetrie im Androecium beginnt in mittleren Stadien. Asymmetrie des Gynoecium beginnt in frühen, oder mittleren bis späten Stadien. In monosymmetrischen und asymmetrischen Kronen von enantiostylen Blüten dienen möglicherweise ausgeprägt konkave und robuste Petalen der Reflexion des Pollenstrahls während der Blütenvibration bei der Buzz-Bestäubung. Vorläufige Rekonstruktionen des ursprünglichen Merkmalszustandes unterstützen nur mässig die Hypothese, dass das Gynoecium in den Vorfahren von *Senna* nicht aus der Medianebene gekrümmt war, aber sie unterstützen stark die Hypothese, dass asymmetrische Androecien und Kronen von enantiostylen Blüten enstanden sind.

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